Parechovirus central nervous system infection in neonates and young children

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Parechovirus central nervous system infection in neonates and young children

A 5-year follow-up

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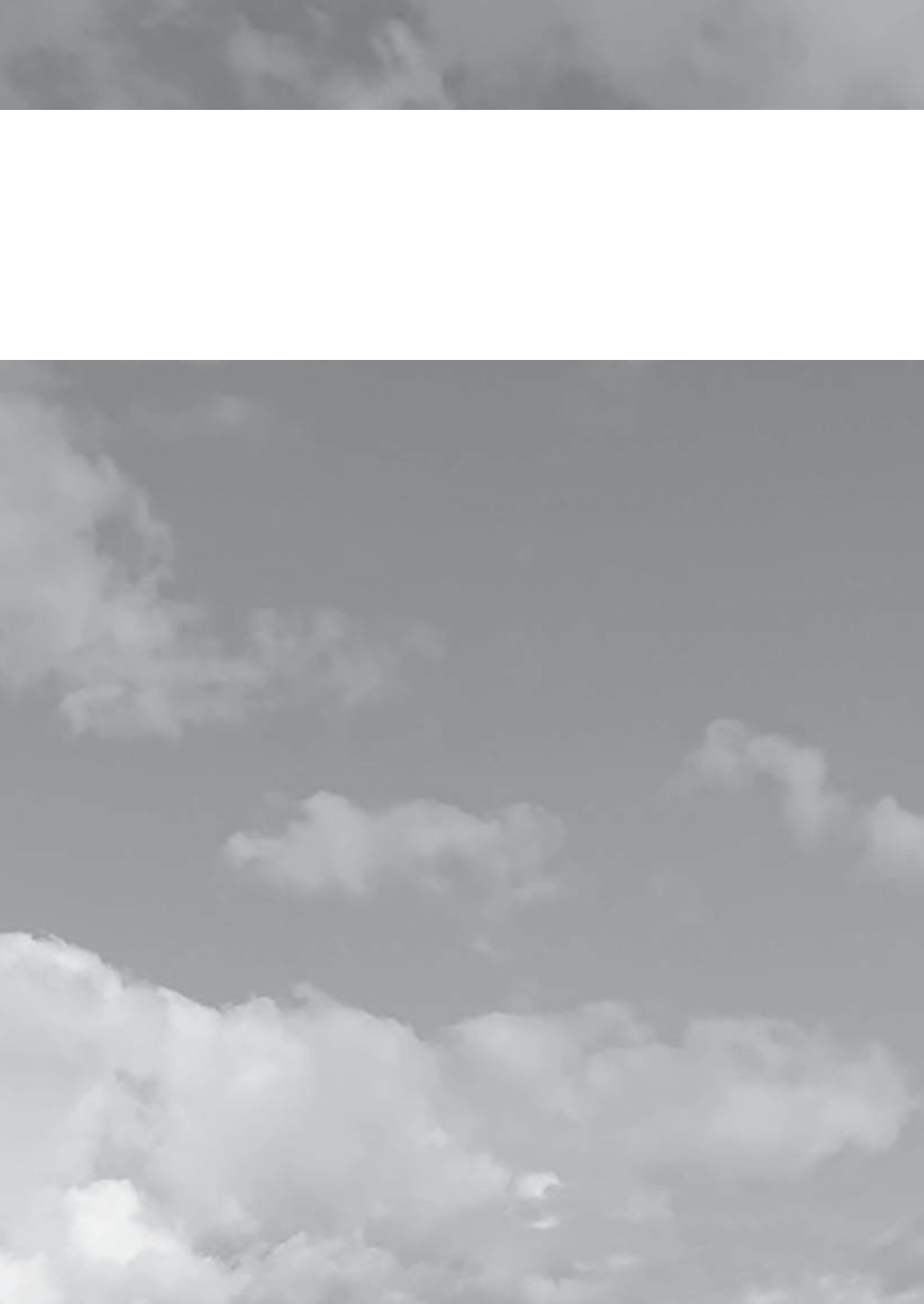

Part 1

General introduction

1

General introduction

Introduction

Parechovirus-A, formerly named human Parechovirus (PeV-A)[1] is the second most common cause of viral meningitis in children next to Enterovirus (EV). There is a paucity of cohort studies that have prospectively evaluated short- and long-term neurodevelopmental outcome in children with PeV central nervous system (CNS) infection. In this thesis, we present various studies that provide insight into the adverse neurologic and neurodevelopmental outcomes of children with an PeV-CNS-infection in the first 5 years after the onset of the infection. We discuss and provide suggestions for management of follow-up.

Viral meningitis

Viral infiltration of the CNS can result in various clinical syndromes, including meningitis[2,3] encephalitis,[4] and meningoencephalitis.[5] Encephalitis[4] and meningoencephalitis[5] are the most severe manifestations of viral CNS-infection. As abovementioned, viral meningitis is frequently caused by EV and PeV.[6,7]

Definitions

Viral meningitis: like in bacterial meningitis, viral meningitis in neonates and young children is characterised by an abrupt onset of fever accompanied by non-specific symptoms such as irritability, poor feeding, vomiting, diarrhoea, rash and respiratory symptoms. Physical abnormalities include bulging fontanel and neck stiffness.[2]

Encephalitis: an inflammation process involving the brain parenchyma. It is associated with neurologic dysfunction and causes altered mental status early in the course, and focal or diffuse neurological signs may be present.[4] It can cause direct damage to the white or grey matter resulting in neurologic dysfunction.[8]

Meningoencephalitis: a CNS-infection with clinical features of both meningeal and parenchymal disease.[5]

In daily practice, especially in neonates, it is not easy to distinguish meningitis from encephalitis, even for an experienced paediatrician. Therefore, in this thesis we will use the term CNS-infection.

Clinical presentation

Clinical suspicion of CNS-infection

Most young children with CNS-infection present with fever, irritability, feeding problems, rash and/or seizures.[9,10] Neonates often present with bulging fontanel and/or other a-specific symptoms such as decreased activity, inconsolable crying or irritability during changing of posture and handling. Older children complain more often about headache. They may also present with meningeal irritation such as photophobia and neck stiffness. This is often accompanied by nausea and vomiting.[6,11]

Diagnose of PeV-CNS-infection

The clinical presentation of PeV-CNS-infection is similar to that of EV-CNS-infection. It is not possible to differentiate between the two based only on clinical symptoms. The diagnose of PeV-CNS-infection is based on the combination of uneventful medical history, clinical suspicion of CNS-infection and confirmation of positive PeV reverse transcriptase quantitative real-time polymerase chain reaction (RT-qPCR) in the cerebrospinal fluid specimen.[12-15] With the introduction of RT-qPCR in 2008, the accuracy of the diagnostic methods for PeV improved substantially. It is superior to serology and culture in different body specimens, including cerebrospinal fluid, blood, nasopharyngeal aspirate, urine and faeces.[13,16,17] This has made it possible to more accurately distinguish an EV- and PeV-infection in daily practice.

History

PeV belongs to the same family of Picornaviridae as EV.[18] PeV and EV are single-stranded Ribonucleinases (ssRNA) viruses. PeV was formerly known as enterovirus ECHO types E22 and E23. These types were found to have fewer structural proteins than EVs and were genetically different in the Viral Protein 1 coding region (VP1). Therefore, they were reclassified as PeV in the new genus Parechovirus.[19] The E22 and E23 were renamed human parechovirus 1 and 2 (PeV-A1 and PeV-A2). In 2014, the genus of PeV was subdivided into four species: PeV-A-D. Only PeV-A infect humans.[11] At present, there are 19 known genotypes of human PeV-A: PeV-A1-19.[11,18] PeV-B, -C and -D infect animals.

Incidence and prevalence

Currently there is a European Network for Non-Polio Enteroviruses (ENPEN) for surveillance and data sharing.[20,21] Viral meningitis[3] is common and often goes unreported in large databases for research. Accurate incidence and prevalence numbers of PeV-CNS-infection are unknown.

The incidence is the number of new cases with a specific disease in a specific period. The combination of a scarcity of robust incidence data and the broad spectrum of clinical presentations of neonatal PeV-CNS-infection makes it difficult to determine its real incidence. Most data on PeV-CNS-infection are based on single centre hospital-based studies that routinely tested for PeV.[11]

Prevalence refers to the number of cases who have a specific disease at a specific point in time or during a specific period. PeV-A3 is listed in the distribution of the 15 most reported EV- and PeV-types per year by the United States National Enterovirus Surveillance System, 2014–2016. The prevalence of PeV-A3 was estimated to be 2.3%. For whom the age was known, the age-range was 1–10 years with a median age of 4 years.[22] A recent PeV-A3 seroepidemiology study from Australia, the Netherlands, and the United States, based on neutralizing antibodies in the populations, suggests that PeV-A3 infection is common and occurs in 11% of children aged < 1 year.[23] The overall prevalence of neutralizing antibodies to PeV-A3 increases with nearly 33% in children aged 1–2 years.[23] The average age of women having their first child in most Western countries is more often > 30 years. In those countries the prevalence of neutralizing antibodies to PeV-A3 declined to 42% in those older than 30 years. This may be the reason why they lack adequate protective maternal PeV-3 antibodies to pass on to their children. This is supported by the observation that only 33.8% of the children < 1 year of age had sufficient protective maternal antibodies.[23]

Clinical relevance

PeV-CNS-infections are frequent causes of severe disease and intensive care admission of neonates and young children aged ≤ 3 months,[7,24-26] especially the types PeV-A1 and PeV-A3.[26] PeV-A1 is more often associated with gastro-intestinal tract and/or respiratory tract involvement than with CNS-infections.[26-28] The more neurotropic strains of PeV-A3 often cause CNS-infection and sepsis like illness in neonates and young children.[14,25,29,30]

Pathogenesis of PeV-A3 CNS-infection and white matter injury

Transmission and replication

Transmission of PeV seems to occur primarily through the faeco-oral route. Transmission by respiratory and transplacental routes are less frequent.[11] Primary replication of the virus occurs mainly in the epithelial cells of the oropharyngeal and intestinal mucosa. Some replication occurs in the nasopharynx, from where the virus may spread to the upper respiratory tract lymphatics and result in pulmonary inflammation (pneumonia). If swallowed, the virus reaches the stomach and lower gastro-intestinal tract, where it binds to specific receptors and results in gastrointestinal inflammation (gastroenteritis).[26,31] When it enters the bloodstream, it causes viremia and can be transported to the rest of the body. In this way it can potentially infect other organs such as the skin (exanthems), heart (myocarditis), pleura (pleurodynia), muscles and nerves (acute flaccid paralysis) and CNS (meningitis or encephalitis).[11]

Compared to other PeV types, PeV-A3 strains show faster replication in neural cells and PeV-A3 receptor seems to facilitate the entry of the virus into neonatal CNS cells.[32]

Regulating of innate immune response against PeV-A3

The innate immune system responds rapidly to viruses by inducing the production of cytokines. Toll-like receptors (TLR)-7 and TLR-8 play an important role in regulating of innate immune response against PeV-A3.[33] After endocytosis, the PeV-A3 releases its ssRNA. The ssRNA binds to the TLR-7 and TLR-8 and initiates the innate immune response. Subsequently reactive inflammatory and regulatory cytokines are produces by macrophages. Once it arrives inside the neuronal cell body, the developing axon and the growth cone, it may result in inhibition of axonal growth and neuronal apoptosis.[33] A recent study published in 2020 reports on antibody production which clears viruses in the infected CNS-cells and regulates the inflammation. This is based on co-operation between the innate and adaptive immune system.[34]

Neurons are non-renewable cells. The transmission of PeV-A3 stimulates the production of destructive effects in the developing brain structures in a phase in which axonal growth and neurodevelopment are very active.[35-37]

Effect of the PeV-A3 infection on neonatal CNS cells

When the PeV-A3 breaks through the blood-brain barrier and reaches the brain, it may result in white matter damage[14,33,38-41] and the ensuing extensive subcortical white matter damage. It can also cause damage to grey matter regions. The neurologic damage caused by PeV in the CNS may be temporary or permanent,[14,38-41] and can subsequently lead to neurologic sequelae and neurodevelopmental disorders, such as neurologic function abnormalities, impairment in auditory and visual functions, gross motor function (GMF) neurodevelopmental delay, fine motor function (FMF) neurodevelopmental delay, cognitive impairment, behavioural- and emotional/personal-social problems and speech- and language delay.[10,14,42-46]

Therapy management

Antiviral therapies

At present, the management of PeV-A3-CNS-infection includes early recognition and supportive care. Despite the high occurrence of PeV-infection, there are currently no antiviral drug therapies (or vaccine) such as specific antibody therapy against PeV-A3.[47] Successful antibody-based therapy with high dose of intravenous immunoglobulin has been described in a child with dilated cardiomyopathy caused by PeV-A1.[48] A very recent study suggests that Posaconazole may contribute to the development of a potential antiviral drug with an antiviral activity against PeV-A3.[49]

Early GMF-neurodevelopmental intervention

Neurodevelopmental disorders are conditions that interfere with CNS-development at an early age.[50] Young children with neurodevelopmental delay often have problems with their postural control, an important component of GMF-neurodevelopment in the first year of life.[51,52] GMF-neurodevelopmental delay at a young age might be a prelude for neurodevelopmental disorder, later in life.[53] It is suggested that different pathophysiological processes in the young brain may result in neuromodulatory mechanisms that can negatively or positively influence the neuroplasticity of the developing brain. These effects seem to depend on the type of trigger and the age of onset. As these neuromodulatory mechanisms occur during sensitive developmental periods, they can strongly modulate the neuronal circuits.[36,37] This suggests that early neurodevelopmental intervention may result in positive changes in neurological patterns.[37] Early detection of GMF-neurodevelopmental delay is

therefore essential for appropriate early GMF-neurodevelopmental interventions, such as paediatric physical therapy.[53,54] It is generally accepted that early GMF-neurodevelopmental interventions should be started, preferably in the period of rapid neural development.[36,37] This may have a positive effect on the generalized neurodevelopmental outcomes and prevent permanent impairment after a PeV-CNS-infection. GMF-neurodevelopment, an early sign of a generalized neurodevelopmental disorder, is the most important outcome of this thesis.

Motor neurodevelopmental assessment tests

The characteristics of the motor neurodevelopmental assessment tests

In the current studies, we assessed the GMF-neurodevelopment of participating children with the Alberta infant motor scale (AIMS),[55,56] the Bayley scales of infant and toddler development version-3 (Bayley-3-NL)[57-59] and the Movement assessment battery for children version-2 (M-ABC-2 NL).[60, 61] These three norm-referenced observational- and performance-based instruments are designed to monitor the GMF-neurodevelopment in children at risk of CNS disorder. We also tested the FMF-neurodevelopment in children of 24 months and older using the Bayley-3-NL and the M-ABC-2 NL. The AIMS is standardized for Canadian children but is also suitable for cross-cultural use.[56] Dutch studies from 2007 and 2019 showed that the percentile scores of Dutch children were significantly lower than the scores of the Canadian population standard norm. They reported that 75% of the Dutch children scored below the 50th percentile.[62,63] The sequence of gross motor milestones was, however similar between Dutch and Canadian children, indicating that Dutch children only acquired gross motor milestones at an older age. We used an AIMS *Z*-score of ≤ -1.30 as threshold for suspect GMF-neurodevelopmental delay when we compared the GMF of participating children to the population standard norm (equivalent to the 10th percentile of the AIMS).[56,64] As from 2014, the Bayley-3-NL scores have been standardized for Dutch children. A comparison study of Bayley-3-USA and Dutch-norm scores showed that more Dutch children were classified as having a neurodevelopmental delay when the USA population standard norm was used than when the Dutch population standard norm was used.[59] For our cohort, we were able to convert the Bayley-3-USA raw scores, measured before 2014, into the Dutch standard norm scores.[58] The M-ABC-2 NL was released in 2010 and is based on the Dutch population standard norm.[60,61] Within the group of children with PeV-CNS-infection, we were able to test within one age-band (3–6 years) of the movement assessment battery for children version-2 (M-ABC-2-NL). Switching age-bands can cause discontinuity in the outcome.[65,66]

Motor neurodevelopmental assessment over 5 years of follow-up

All three motor neurodevelopmental assessment tests have shown the ability to detect subtle deviations in motor performance (minor motor delays). The AIMS is the most appropriate test for GMF-assessment in children in the age range 4 to 15 months.[67] We used the Bayley-3-NL and the M-ABC-2-NL tests for the assessment of GMF- and FMF-neurodevelopment in children of respectively 2 and > 4 years of age. The motor neurodevelopmental assessment tests overlap for some age-ranges. For the assessment of children aged 6–15 months the AIMS is more suitable to detect minor GMF-neurodevelopmental delays (especially postural control) than the Bayley-3-NL.[67] For children aged 36–42.5 months, the Bayley-3-NL tasks are easier to carry out than those of the M-ABC-2-NL.[60] To compare all test results cross-sectionally and longitudinally, we converted the raw or scale scores of the GMF- and FMF-neurodevelopment into *Z*-scores and interpreted results according to the technical manual of each instrument.[55,57,58,60]

The characteristics of the motor neurodevelopmental assessment tests used in the current study are summarized in Table 1.1.

Aims and outline of this thesis

Aims

- We investigated the longitudinal association between PeV-CNS-infection and GMF-neurodevelopmental outcome of neonates and young children from 6 months to 5 years after the infection. We compared these children with the population standard norm and with other subgroups: children with EV-CNS-infection, with PeV-CNS-infection-elsewhere in the body (not in CNS), with EV-CNS-infection-elsewhere in the body (not in CNS) and with a reference group: peers in whom no pathogen was detected.
- We also investigated the longitudinal association between PeV-CNS-infection and FMF-neurodevelopmental outcome of neonates and young children from 2 to 5 years. We compared the children with PeV-CNS-infection with the population standard norm and with the other above-mentioned subgroups.
- Additionally, we critically appraised and analysed the existing literature on the neurologic and neurodevelopmental outcomes of neonates and young children with PeV-CNS-infection.

Table 1.1: Characteristics of motor neurodevelopmental assessment tests used in the current study during 5-year follow-up

	Year of release	Age range	Age range in current study	Population standard norm	Test domains	Test domains used in current study	Suitable for detecting minor motor delay	Discriminatory and valid	Suitable for cross-cultural use	Raw scores / Items based	Compare with population standard norm: *Z*-score threshold
AIMS	1994	0–19 months	6–15 months	Canadian children	GMF	GMF	Yes, GMF floor effect < 4 months; ceiling effect > 15 months[56]	Yes	Yes[56]	Higher raw score indicates more mature GMF**	≤ -1.30*
Bayley-3-NL	2014	16 days to 42.5 months	> 15–42.5 months	Dutch children	GMF, FMF, cognition, language social-emotional and adaptive skills	GMF and FMF	Yes, GMF and FMF no ceiling effect[68]	Yes	Yes Different norms geo-graphical	Higher raw score indicates more mature GMF and FMF**	≤ -1.00
M-ABC-2-NL	2010	3–16 years	> 42.5 months–5/6 years	Dutch children	GMF (static & dynamic balance), FMF (manual dexterity), aiming & catching	GMF (static & dynamic balance) and FMF (manual dexterity)	Yes, GMF and FMF ceiling effect domain aiming & catching[69]	Yes	Yes Different norms geo-graphical	Timed item scores and number of correct scores	≤ -1.00

* Equivalent to the 10th percentile of the population standard norm.
** Number of test items credited as passed.
Abbreviations: AIMS: Alberta infant motor scale; Bayley-3-NL: the Bayley scales of infant and toddler development version-3; FMF: fine motor function; GMF: gross motor function; M-ABC-2 NL: the movement assessment battery for children version-2.

Outline

Chapter 1 contains the general introduction wherein we describe the history and pathogenesis of PeV-CNS-infection, the clinical presentation and assignment of the children into different subgroups. We describe the motor neurodevelopmental assessment tests we used in the 5-year follow-up and we highlight the importance of early GMF-neurodevelopmental intervention.

Chapter 2 presents a systematic review and meta-analyses of neurodevelopmental outcomes in neonates and young children with PeV-CNS infection. The primary outcomes are neurologic sequelae, impairment in auditory or visual functions, and GMF-neurodevelopmental delay. The secondary outcomes are signs of late neurodevelopmental delay such as FMF-neurodevelopmental delay, cognitive impairment, behavioural- and emotional/personal-social problems and speech- and language delay. With this systematic review, we attempted to close the information gap for clinicians and neurodevelopmentalists of existing literature by conducting meta-analyses. We hope it'd assist them in making decisions on frequency and duration of follow-up as well as strategies to mitigate potentially evolving sequelae and neurodevelopmental delay after PeV-CNS-infection.

In the study described in **Chapter 3**, we performed a prospective investigation of short-term GMF-neurodevelopmental outcomes in neonates and young children, 6 months after PeV-CNS-infection. PeV-CNS-infection is defined as the combination of clinical symptoms of meningitis and positive PeV RT-qPCR in cerebrospinal fluid. The children with PeV-CNS-infection were compared to children without CNS-infection but a positive PeV RT-qPCR in at least one other body specimens: blood, nasopharyngeal aspirate, urine and faeces (PeV-infection-elsewhere) and to peers in whom no pathogens were identified (reference-group). Finally, we compared their outcomes to the population standard norm. For the GMF-neurodevelopmental delay assessment, we used the AIMS assessment test.

Then, **Chapter 4** presents a study in which we compared the GMF- and FMF-neurodevelopmental outcome in children aged 2 days to 12.8 years. A group of children with a PeV- or EV-CNS-infection was compared to a group of children with a PeV- or EV-infection-elsewhere and with children in whom no pathogen was identified (reference-group). Twenty-four months after the presentation, the children were assessed with the Bayley scales of infant and toddler development-3 and the M-ABC-2-NL.

In **Chapters 5 and 6** we show the longitudinal association of GMF- and FMF-neurodevelopmental outcomes of neonates and young children with PeV-CNS-infection. We used the

AIMS, the Bayley-3-NL and the M-ABC-2-NL to assess the motor neurodevelopment of participants. In **Chapter 5**, we compared children with PeV-CNS-infection with peers in whom no pathogens were identified (reference-group) and with the population standard norm during follow-up between 6 and 24 months after infection. In **Chapter 6**, we compared the group of children with PeV-CNS-infection with an age of onset under 3 months to peers with EV-CNS-infection and to the population standard norm. We followed them up at 6, 12 and 24 months, and at 5 years after the onset of the infection. In both longitudinal studies, the analysis included linear mixed models with adjustment for confounders and effect modifiers, such as age at onset, maternal education, time from infection and gender.

In **Chapter 7**, the general discussion, we reflect on the findings of our studies, and in addition discuss the strengths and limitations. We also discuss their implications for clinical practice and recommend strategies for future research projects.

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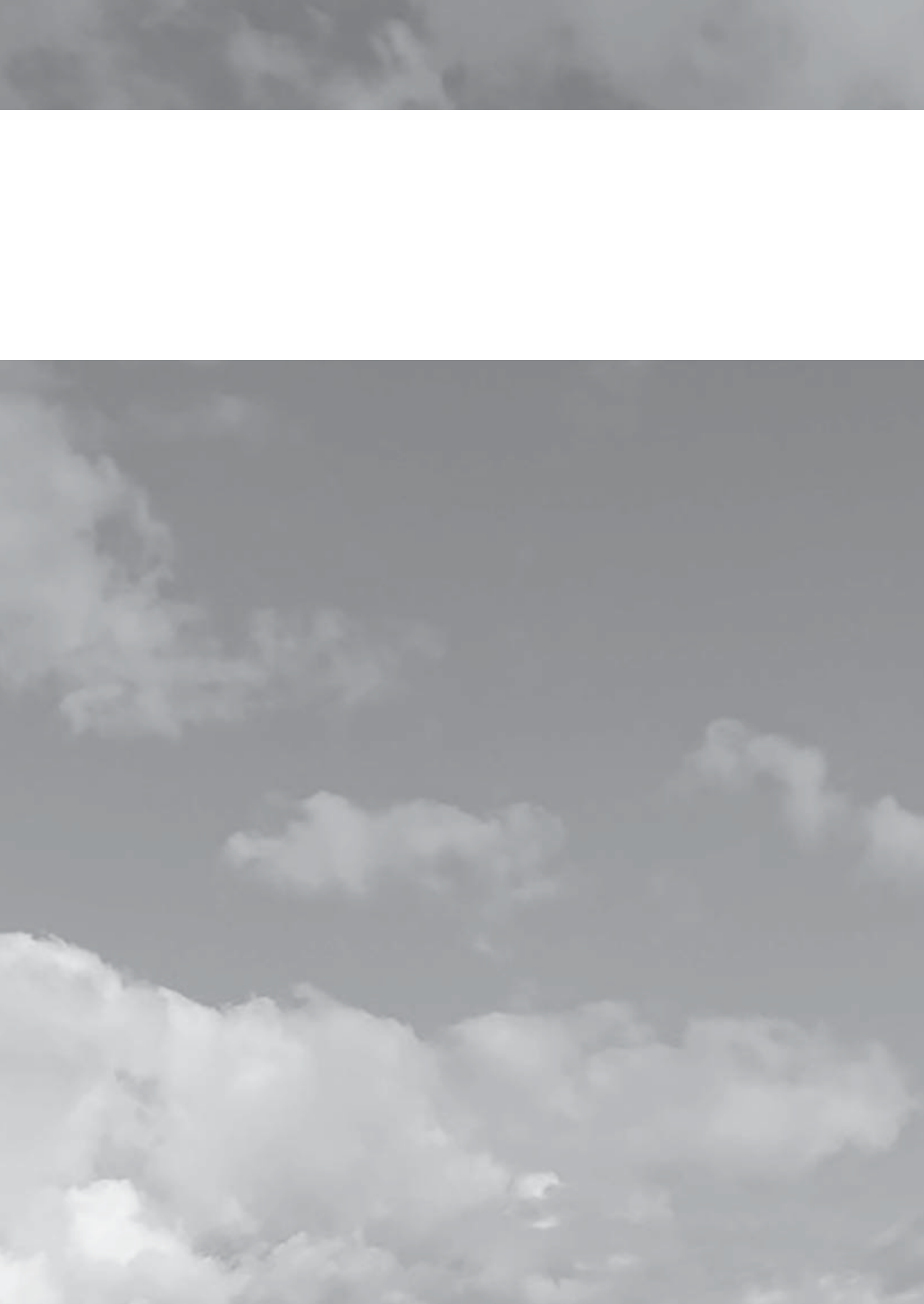

Part 2

Literature search Parechovirus CNS-infection and neurologic and neurodevelopmental outcome

2

Neurological and neurodevelopmental outcomes after human Parechovirus CNS-infection in neonates and young children: a systematic review and meta-analysis

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Abstract

Background: Human parechoviruses are a major cause of CNS-infection in neonates and young children. They have been implicated in neurological sequelae and neurodevelopmental delay. However, the magnitude of this effect has not been systematically reviewed or assessed with meta-analyses. We investigated short-term, medium-term, and long-term neurological sequelae and neurodevelopmental delay in neonates and young children after parechovirus-CNS-infection.

Methods: In this systematic review and meta-analyses of studies, we searched PubMed, Embase, and PsycInfo, from the inception of the database until March 18, 2019, for reviews, systematic reviews, cohort studies, case series, and case control studies reporting on neurological or neurodevelopmental outcomes of children 3 months or younger with parechovirus infection of the CNS. Studies that were published after Dec 31, 2007, assessed children younger than 16 years, detailed parechoviruses infection of the CNS (confirmed by PCR), and followed up on neurological and neurodevelopmental outcomes were included. Studies published before Dec 31, 2007, were excluded. The predefined primary outcomes were the proportions of children with neurological sequelae, impairment in auditory or visual functions, or gross motor function delay. The proportion of children in whom neurological or neurodevelopmental outcomes were reported was pooled in meta-analyses. For each outcome variable we calculated the pooled proportion with 95% CI. The proportion of children in whom neurological or neurodevelopmental outcomes were reported was extracted by one author and checked by another. Two authors independently assessed the methodological quality of the studies.

Findings: 20 studies were eligible for quantitative synthesis. The meta-analyses showed an increasing proportion of children with neurological sequelae over time: 5% during short-term follow-up (pooled proportion 0.05 [95% CI 0.03–0.08], $I^2 = 0.00\%$; $p = 0.83$) increasing to 27% during long-term follow-up (0.27 [0.17–0.40], $I^2 = 52.74\%$; $p = 0.026$). The proportion of children with suspected neurodevelopmental delay was 9% or more during long-term follow-up. High heterogeneity and methodological issues in the included studies mean that the results should be interpreted with caution.

Interpretation: This systematic review suggests the importance of long follow-up, preferably up to preschool or school age (5–6 years), of children with parechovirus infection of the CNS. Although not clinically severe, we found an increasing proportion of neonates and young children with CNS-infection had associated neurological sequelae and neurodevelopmental delay over time. We recommend the use of standardised methods to assess neurological and neurodevelopmental functions of these children and to compare results with age-matched reference groups.

Introduction

Parechoviruses can cause gastrointestinal or respiratory illness in neonates and young children (< 3 months old), and have been implicated in cases of myocarditis in adults and children. Since their first description in 1956,[1] parechoviruses have become the second most common cause of CNS-infection in childhood.[2,3] Use of molecular diagnostic methods has substantially increased their isolation in body specimens, including cerebrospinal fluid.[4-6] Parechoviruses have been implicated in brain periventricular white matter damage in young children,[7-10] who are at a higher risk of severe disease and admission to the intensive care unit.[7-9,11-15] Despite different reports of permanent,[7,9] temporary,[16] and no[17,18] neurological sequelae or motor and cognitive impairment after parechovirus infection of the CNS, the magnitude of this effect has not been systematically reviewed or assessed with a meta-analyses.

Early signs of parechovirus infection of the CNS associated with generalised neurodevelopmental delay, such as neurological abnormalities, impairment of auditory and visual functions, and gross motor function delay, are often detected in the first year of life.[19] Milder disabilities in other developmental domains, such as fine motor developmental, cognition, behaviour, and speech and language development, might manifest only years later.[20,21] Early detection of these neurodevelopmental abnormalities is essential for appropriate developmental interventions.[19,22,23]

The purpose of this systematic review and meta-analysis was to critically appraise the existing literature on the neurological and neurodevelopmental outcomes of neonates and young children after parechovirus infection of the CNS.

Methods

Search strategy and study selection

This review followed the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA)-guidelines.[24]

In this systematic review and meta-analysis, we searched the PubMed, Embase, and PsycInfo databases for reviews, systematic reviews, cohort studies, case series, and case control studies published from the inception of each database until March 18, 2019. The search terms included "picornaviridae" OR "parechovirus" AND "children" OR "infants" OR "newborns" and "meningitis" OR "central nervous system". The full search strategy is reported

in Appendix 2.1. To not omit any relevant data, we examined the reference lists of articles, searched the grey literature, and contacted corresponding authors for unpublished data. We screened the full text of papers for inclusion. We restricted inclusion to papers published after Dec 31, 2007 because the diagnostic methods used for parechovirus infection of the CNS (serology and culture) before 2008 were not sensitive and specific enough to differentiate it from enterovirus infection. We included case-control studies and observational case-series on neurological and neurodevelopmental outcomes in children after parechovirus infection of the CNS (e.g., meningitis,[25] encephalitis,[26] meningoencephalitis,[27] and sepsis-like illness[28]), confirmed by positive PCR (parechovirus-qPCR).[4,5,29] In the meta-analysis, we included single-group and multigroup studies. From the multi-group studies (e.g., children affected by enterovirus described side-by-side with children affected by parechovirus) only data from the children affected by the parechovirus were included in the meta-analyses. We excluded case reports. Two independent authors (TMTvH and CCO) reviewed the titles and abstracts, with a third acting as adjudicator (RGE) to solve disputes.

Data analysis

The predefined primary outcomes were the proportions of children with neurological sequelae, impairment in auditory or visual functions, or gross motor function delay. The secondary outcomes were the proportions of children with late neurodevelopmental delay (i.e., fine motor function delay; cognitive impairment; behavioural, emotional, and personal-social problems; and speech and language delay).

We entered descriptive and quantitative data into a standardised data collection form detailing four different areas. First, study characteristics (first author, year and country of publication, and recruitment period). Second, patients' characteristics (clinical setting, number of children, age at onset, and gestational age). Third, diagnostic methods for identification of parechovirus infection of the CNS (clinical symptoms, molecular diagnosis with parechovirus-qPCR of the cerebrospinal fluid and other body-specimens, and supportive diagnostic methods, such as neuroimaging [brainMRI, cranial ultrasound, or CT scan] or electroencephalography). Finally, the results of neurological and neurodevelopmental domains assessed during follow-up. One author (TMTvH) collated and entered the results in database (Mircosoft Excel 2016) and a second (RGE) checked it for omissions and mistakes.

Two authors (TMTvH and RGE) independently assessed the methodological quality of each study. To evaluate the risk of bias in studies describing the proportions of children with the

primary or secondary outcomes in a single study group, we assessed the design (selection, ascertainment of diagnosis and outcomes, follow-up, and reporting) of each study based on the tool for evaluating the methodological quality proposed by Murad and colleagues.[30] We assessed the objectives of each study, the characteristics of the individuals included, and other factors (such as age at onset, gestational age, coinfection, and intensive care unit admission) that could adversely influence the outcomes of interest.

We subdivided the duration of follow-up into short term (≤ 6 weeks), medium term (6–12 months), and long-term (≥ 12 months), depending on when outcomes were assessed after diagnosis. These outcome timepoint categories were agreed upon for use in this study following the identification of papers on pragmatic grounds and influenced by the outcomes observed. The follow-up timepoints are not continuous, with no studies having follow-up data between 6 weeks and 6 months, and were determined post hoc. We took into account the difficulty of differentiating the effects of generalised illness or hospitalisation on the neurological and neurodevelopment of young children from the sequelae of the viral infection during a short follow-up period.[31] However, a follow-up period of 6 weeks to 6 months is too short to detect most neurological sequelae or neurodevelopmental delay that manifest later.[19-21] Longitudinal studies detecting mild, late-onset neurological and neurodevelopmental abnormalities after 12 months or more support this assumption.[19-21] On the basis of this we created the medium-term and long-term follow-up categories.

Statistical analysis

We used R (version 3.6.3) for the statistical analysis, with the meta-analyses packages Matrix (version 1.2-18)[32] and Metafor (version 2.1-0).[33,34] The proportion of children with each outcome of interest was expressed with 95% CIs. For studies reporting on outcomes of interest in more than two categories, we followed the manufacturer's instructions in applying a cutoff value of 1.0 standard deviation (*SD*). We dichotomised study children either as being within the norm (> 1.0 *SD* from the age-adjusted norm) or as being below the norm (≤ -1.0 *SD* from the age-adjusted norm).[35-37] We analysed the heterogeneity of studies by comparing clinical characteristics, duration of follow-up, and outcome measures of interest before deciding whether or not it was sensible to pool the results.

We hypothesised that the true proportion of children with an outcome of interest would vary between studies, and used a random effects logistic regression model (generalised linear mixed model) to pool the results in a meta-analysis. An advantage of the generalised linear

mixed model is that it can adjust for zero events.[34] For the forest plots, we used a continuity correction for zero cell counts by adding 0.01 to each count of the studies included in the meta-analysis.[38] If no more than 4 were included in a meta-analysis, we did a sensitivity analysis using a fixed-effect model to test the robustness of the random-effects model.

Statistical heterogeneity was assessed by visual inspection of the forest plots, with the likelihood-ratio and the Wald-tests. We calculated I^2, and assigned the adjectives low (< 25%), moderate (25–75%), or high (> 75%) to I^2 values.[39] For meta-analyses that showed high values for I^2, we plotted the individual study results, but refrained from summarising these as a pooled proportion.

Role of the funding source

No funding was received for this study. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Of 421 records identified, 65 studies were retrieved and assessed. Of these, 45 were excluded (Figure 2.1).[2,8,9,13-15,40-78] 20 studies were included in the systematic review and meta-analyses.[6,7,16,17,79-94]

Table 2.1 presents study and patient characteristics. Most of the included studies were done in Europe (Italy,[88] Norway,[91] Netherlands,[7,16,82,85,93] UK)[6,17,81,92,94] or Australia.[79,80,86,87] The ages at onset of infection ranged between preterm age (< 37 weeks gestational age)[6,7,79,80,86,88,93,94] and 18.5 months.[86] Two studies were done solely in an intensive care unit,[7,88] eight studies were done in both intensive care unit and non-intensive care unit wards,[6,17,79,80,86,87,93,94] and eight studies were done in non-intensive care unit hospitals.[16,81,82,84,85,89,90,92] Reported clinical symptoms at presentation were fever and rash in all studies, irritability in 16 (80%), poor feeding in 13 (65%), seizure and lethargy in 12 (60%) studies, diarrhoea in 11 (55%), respiratory symptoms in ten (50%), abdominal distension and vomiting in eight (40%), tachycardia in four (20%) studies, and circulatory shock in three (15%). 16 studies diagnosed parechovirus infection of the CNS with a positive parechovirus-qPCR in cerebrospinal fluid and at least one other body specimen (e.g., nasopharyngeal aspirate, blood, urine, or faeces).[6,7,16,17,79,80,82,85-91,93,94] Four studies diagnosed it with positive parechovirus-qPCR in cerebrospinal fluid only.[81,83,84,92] Brain MRI imaging was done in 12 studies; in only two

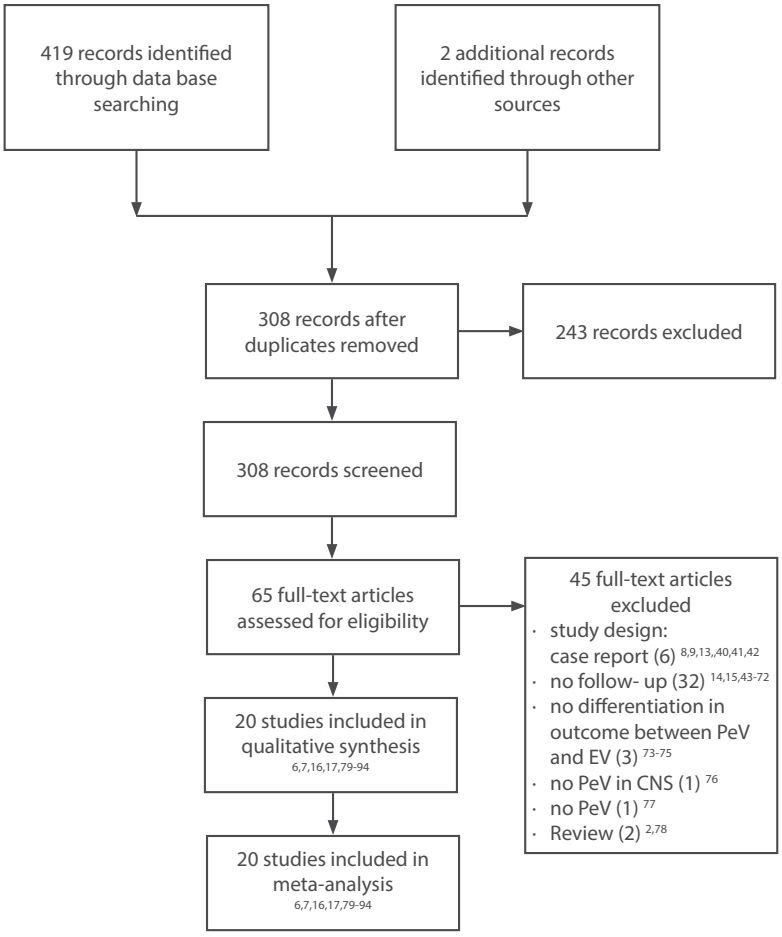

Figure 2.1: Study selection process.
Abbreviations: CNS: Central Nervous System; EV: enterovirus; PeV: human parechovirus.

studies did all the children have an MRI,[7,93] none of the studies did cranial ultrasound or electroencephalography in all children.

The period between diagnosis and assessment of outcomes of interest varied between studies. 11 studies with a total of 290 children assessed outcomes of interest during short-term follow-up,[6,79,82-84,86,88-92] eight with a total of 247 children during medium-term follow-up[7,17,81,82,85-87,89] and nine with a total of 183 children during long-term follow-up.[7,16,79,80,82,86,91,93,94]

Table 2.1: Characteristics of the included studies and study populations

			Characteristics of included children with PeV-CNS-infection				Ascertainment of PeV-CNS-infection		
Author (year)	Country	Recruit-ment	Clinical setting	Number	Mean age (*SD*) at onset of clinical signs, days	Preterm births, *n* (gestational age range in weeks)	PeV-qPCR	Supportive diagnostic methods (*n* abnormal/*N* assessed)	Follow-up duration
Britton et al.[79] (2016)*†	Australia	2013–14	ICU and non-ICU	13	32.2 (26.6)‡	5 (28–35)	CSF or other body specimen	MRI 7/8, cUS 2/7, and EEG 6/7	Neurological outcome at ≤6 weeks; neurological outcome, visual function outcome, and neurodevelopmental outcome at ≥ 12 months
Britton et al.[80] (2018)*†	Australia	2013–14	ICU and non-ICU	44	42.7 (27.8)‡	3 (33–36)	CSF or other body specimen	MRI 3/7, cUS 2/1, and EEG 3/4	Neurodevelopmental outcome at ≥ 12 months
Chakrabarti et al.[81] (2018)	UK	2012–16	Non-ICU	14	36.4 (37.0)‡	Not reported	CSF	No MRI, cUS, or EEG	Neurological outcome at 6–12 months
De Jong et al.[82] (2017)	Netherlands	2011–12	Non-ICU	4	10.0 (8–29)§	0	CSF or blood	MRI 0/3, cUS 0/3, and no EEG	Neurological outcome and auditory function outcome at ≤ 6 weeks, neurological outcome at 6–12 months, and neurological outcome and neurodevelopmental outcome at ≥ 12 months
Ferreras et al. (2018)¶†	UK	2016	ICU and non-ICU	106	32 (3–150)§	8 (gestational age not reported)	CSF or other body specimen	MRI 2/7, cUS 0/10, and no EEG	Neurological outcome and auditory function outcome at ≤ 6 weeks
Ghanem-Zoubi et al.[83] (2013)	Israel	2007–09	Not reported	13	32.8 (21.8)‡	Not reported	CSF	No MRI, cUS or EEG	Neurological outcome at ≤ 6 weeks

Han et al.[84] (2013)	South Korea	2011–12	Non-ICU	12	54.0 (3–120)‖	Not reported	CSF	No MRI, cUS or EEG	Neurological outcome at ≤ 6 weeks
van Hinsbergh et al.[85] (2019)**	Netherlands	2008–11	Non-ICU	9	31.0 (17.2)‡	0	CSF and other body specimen	No MRI, cUS or EEG	Gross motor function outcome at 6–12 months
van Hinsbergh et al.[16] (2019)**	Netherlands	2008–11	Non-ICU	11	39.0 (29.0)‡	0	CSF and other body specimen	No MRI, cUS or EEG	Gross motor function outcome at ≥ 12 months
Joseph et al.[86] (2019)†	Australia	2013–16	ICU and non-ICU	142	35.0 (6–567)‖	13 (gestational age not reported)	CSF or other body specimen	MRI 15/20, cUS 1/14, and no EEG	Auditory function outcome at ≤ 6 weeks, neurological outcome at 6–12 months, and neurological outcome and visual function outcome at ≥ 12 months
Kadambari et al.[17] (2019)	UK and Ireland	2014–15	ICU and non-ICU	35	34 (15–53)§	Not reported	CSF or other body specimen	No MRI or EEG, and cUS 10/35	Neurologic outcome at 6–12 months
Khatami et al.[87] (2015)*	Australia	2013–14	ICU and non-ICU	118	39.0 (4–285)‖	Not reported	CSF or other body specimen	MRI 7/11, cUS 0/17, and no EEG	Neurological outcome at 6–12 months
Piralla et al.[88] (2014)	Italy	2010–13	ICU	3	all < 30.0††	0	CSF or blood	No MRI, cUS, or EEG	Neurological outcome at ≤ 6 weeks
Renaud et al.[89] (2011)	North America	2009–10	Non-ICU	12	27.5 (19.3)‡	Not reported	CSF and blood	MRI 1/ number not reported and no cUS or EEG	Neurological outcome at ≤ 6 weeks and at 6–12 months

Table 2.1 continues on next page.

Table 2.1: *Continued*

			Characteristics of included children with PeV-CNS-infection				Ascertainment of PeV-CNS-infection		
Author (year)	Country	Recruit-ment	Clinical setting	Number	Mean age (*SD*) at onset of clinical signs, days	Preterm births, *n* (gestational age range in weeks)	PeV-qPCR	Supportive diagnostic methods (*n* abnormal/*N* assessed)	Follow-up duration
Sano et al.[90] (2018)	Japan	2010–15	Non-ICU	11	32.0 (15–42)[§]	Not reported	CSF or other body specimen	No MRI, cUS, or EEG	Neurological outcome at ≤ 6 weeks
Skram et al.[91] (2014)	Norway	2011–11	Not reported	15	22.8 (17.1)[‡]	0	CSF or other body specimen	MRI 2/3, cUS number not reported, and EEG 1/15	Neurological outcome at ≤ 6 weeks and gross motor function outcome at ≥ 12 months
Tang et al.[92] (2016)[‡¶]	UK	2016	Non-ICU	26	35.0 (8–197)[‖]	Not reported	CSF	MRI 1/1, no cUS, and EEG 1/1	Neurological outcome at ≤ 6 weeks
Verboon-Maciolek et al.[7] (2008)[†‡‡]	Netherlands	1997–2008	ICU	10	25.5 (29.7)[‡]	4 (25–41)	CSF or other body specimen	MRI 9/10, cUS 9/10, and EEG 2/9	Neurological outcome at 6–12 months; neurological outcome, visual function outcome, neurodevelopmental outcome at ≥ 12 months
Verboon-Maciolek et al.[93] (2008)[†‡‡]	Netherlands	1994–2006	ICU and non-ICU	11	8.0 (1–90)[‖]	4 (25–42)	CSF or other body specimen	MRI 8/11, cUS 8/11, and no EEG	Neurological outcome and neurodevelopmental outcome at ≥ 12 months
Vergnano et al.[94] (2015)[†]	UK	2008–12	ICU and non-ICU	50	All < 90.0 days[††]	6 (28–42)	CSF or other body specimen	MRI 10/12 and no cUS or EEG	Neurological outcome, visual function outcome, and neurodevelopmental outcome ≥ 12 months

Abbreviations: CSF: cerebrospinal fluid; cUS: cranial ultrasound; EEG: electroencephalography; ICU: intensive care unit (neonatal or paediatric); qPCR: quantitative polymerase chain reaction.
* Possible overlap between these cohorts; [†] Not corrected for preterm birth; [§] Expressed as median (IQR); [‡] Expressed or calculated as mean (*SD*); [¶] Possible overlap between these cohorts; [‖] No *SD*, but range was reported; ** Possible overlap between these cohorts; [††] Calculation of mean not possible; [‡‡] Possible overlap between these cohorts.

Ten studies (50%) were case-series.[6,7,79,80,83,86,87,91,92,94] Only 11 studies clearly defined inclusion and exclusion criteria.[7,16,81,82,84,85,88-90,93,94] Eight studies excluded children with coinfections (viral, bacterial, fungal, or protozoan).[7,16,82,83,85,90,92,93] Three studies did not specify the exclusion of children with neurodevelopmental delay because of congenital malformations,[87] congenital diseases,[88] or underlying complex syndromes.[94] Studies differed in the severity of disease in patients recruited: a few included only children admitted in a neonatal or paediatric intensive care unit,[7,88] others included children in non-intensive care unit wards,[16,81,82,84,85,89,90,92] and others included a mixture of both.[6,17,79,80,86,87,93,94] 11 studies reported lost-to-follow-up data.[7,16,17,79,80,82,85,86,89,91,94]

Only three studies reported missing data and these were completely at random.[16,82,85] The major objectives differed between studies. Ten studies focused mainly on neurological or neurodevelopmental outcomes.[7,16,17,79,80,82,85,86,93,94] The other ten studies focused on incidence or prevalence,[81,83,90] clinical characteristics,[6,81,87-89,91] or detection methods.[6,84,88,92] There was heterogeneity in outcome measurements. A number of studies based their outcomes only on retrospectively completed symptom-based parental[80] or clinician-completed questionnaires.[6,17] Only three studies masked the health-care professional doing the assessment to previous knowledge of the clinical diagnosis of study children.[16,82,85] Assessment tools of neurological and neurodevelopmental functions varied from Glasgow outcome scale,[79] Liverpool outcome score-follow-up,[80] Ages and stages questionnaire 3rd edition,[79,80,86] Bayley scales of infant and toddler development 3rd edition,[16,82] Alberta infant motor scale,[16,85] to Griffith mental developmental scale.[7,93] In four studies, the assessing paediatrician did not use a standardised assessment tool.[86,87,91,94] These differences made it difficult to categorise the outcomes of interest into any of the neurodevelopmental test-domains without creating classification bias. Therefore, we included these results only in the analyses for neurological outcomes. None of the included studies reported their funding sources. Table 2.2 summarises the methodological aspects of studies.

Figure 2.2 shows the outcomes of interest assessed during short-term follow-up. Ten studies in this category that investigated neurological outcomes[6,79,82-84,88-92] found abnormality in between 0%[82-84,88,90,92] and 23%[79] of individuals. Pooled results showed an average of 5% children with neurological sequelae (pooled proportion 0.05 [95% CI 0.03–0.08], I^2 = 0.00%; p = 0.83). None of the three studies that assessed auditory function[6,82,86] found that parechovirus infection of the CNS was associated with hearing loss. None of these studies assessed visual function, gross motor function, or secondary outcomes.

Table 2.2: Assessment of methodological quality

			Selection					Ascertainment of outcome		
Author (year)	Study objective*	Reporting	Recruitment of children	Inclusion criteria	Exclusion criteria	Lost to follow-up, *n/N* (%)	Reasons lost to follow-up	Data collection	Investigators masked for diagnosis outcome assessor	Standardised measurements
Britton et al.[79] (2016)	Outcome	Descriptive, proportion of children with PeV-CNS-infection	Consecutive	Clinical evidence for CNS-infection and hospitalisation	Age at onset of > 90 days	2/13 (15%)	Not reported	Cross-sectional	No	Standard visual functions screening, GOS, and ASQ-3
Britton et al.[80] (2018)	Outcome	Descriptive, proportion of children with PeV-CNS-infection	Parental questionnaire	Clinical evidence for CNS-infection. Contacted 12 months after PeV-CNS-infection: children whose parents responded were included in the study	Age at onset of > 90 days	37/79 (47%)	No response to the questionnaire	Cross-sectional	No	ASQ-3 and LOS-f/up
Chakrabarti et al.[81] (2018)	Incidence	Clinical characteristics, descriptive, proportion of children with EV-CNS-infection and PeV-CNS-infection	Selection of body specimens	Clinical evidence for CNS-infection, CSF samples positive for PeV, and additional positive clinical criteria	Age at onset of > 90 days	Not reported	Not reported	Cross-sectional	No	Neurological assessment
De Jong et al.[82] (2017)	Outcome	Descriptive, proportion of children with EV-CNS-infection and PeV-CNS-infection	Consecutive	Clinical evidence for CNS-infection and hospitalisation	Age at onset of > 90 days and gestational age of < 37 weeks, coinfection	2/4 (50%)	Missing completely at random	Cross-sectional	Yes	Neurological assessment, standard auditory functions screening, and BSID-III

Ferreras et al.[6] (2018)	Outcome	Descriptive, proportion of children with PeV-CNS-infection	Clinician questionnaire, selection of body specimens	Clinical evidence for CNS-infection, hospitalisation, and contacted after PeV-CNS-infection: children whose clinicians responded were included in the study	Age at onset of ≥ 12 months	Not reported	No response to the questionnaire	Cross-sectional	No	Neurological assessment and standard auditory functions screening
Ghanem-Zoubi et al.[83] (2013)	Prevalence	Descriptive, proportion of children with PeV-CNS-infection	Selection of body specimens	Clinical evidence for CNS-infection	Age at onset of > 90 days and coinfection	Not reported	Not reported	Cross-sectional	No	Neurological assessment
Han et al.[84] (2013)	Detection methods	Descriptive, proportion of children with EV-CNS-infection and PeV-CNS-infection	Selection of body specimens	Clinical evidence for CNS-infection, hospitalisation, PeV positive CSF samples, and review of medical records	Age at onset of > 90 days	Not reported	Not reported	Cross-sectional	No	Neurological assessment
van Hinsbergh et al.[85] (2019)	Outcome	Comparative, risk for outcome of children with PeV-CNS-infection, PeV-elsewhere and control	Consecutive	Clinical evidence for CNS-infection and presentation at emergency or outpatient departments	Age at onset of > 10 months and gestational age of < 37 weeks, coinfection	2/11 (18%)	Missing completely at random	Cross-sectional	Yes	AIMS
van Hinsbergh et al.[16] (2019)	Outcome	Comparative, risk for outcome of children with PeV-CNS-infection and controls	Consecutive	Clinical evidence for CNS-infection and presentation at emergency or outpatient departments	Age at onset of > 24 months and gestational age of < 37 weeks, coinfection	2/11 (18%)	Missing completely at random	Longitudinal	Yes	AIMS, Bayley-3-NL, and M-ABC-2-NL

Table 2.2 continues on next page.

Table 2.2: ***Continued***

			Selection					Ascertainment of outcome		
Author (year)	Study objective*	Reporting	Recruitment of children	Inclusion criteria	Exclusion criteria	Lost to follow-up, *n/N* (%)	Reasons lost to follow-up	Data collection	Investigators masked for diagnosis outcome assessor	Standardised measurements
Joseph et al.[86] (2019)	Outcome	Descriptive, proportion of children with PeV-CNS-infection	Selection of body specimens	Clinical evidence for CNS-infection, hospitalisation, PeV positive CSF samples, and review of medical records	No exclusion criteria reported	65/142 (46%)	Not reported	Cross-sectional	No	Neurological assessment, ASQ-3, standard auditory functions screening, standard visual functions screening
Kadambari et al.[17] (2019)	Outcome	Descriptive, proportion of children with EV-CNS-infection and PeV-CNS-infection	Clinician questionnaire	Clinical evidence for CNS-infection and contacted after PeV-CNS-infection: children whose clinicians responded were included in the study	Age at onset of > 90 days	19/35 (54%)	No response to the questionnaire	Cross-sectional	No	Neurological assessment, and standard auditory functions screening
Khatami et al.[87] (2015)	Outcome	Descriptive, proportion of children with PeV-CNS-infection	Based on reviewing of medical records	Clinical evidence for CNS-infection, hospitalisation, and presentation at emergency or outpatient departments	No exclusion criteria reported	Not reported	Not reported	Cross-sectional	No	Neurological assessment
Piralla et al.[88] (2014)	Detection methods	Descriptive, proportion of children with EV-CNS-infection and PeV-CNS-infection	Consecutive	Clinical evidence for CNS-infection and hospitalisation	Age at onset of > 90 days and gestational age of < 37 weeks	Not reported	Not reported	Cross-sectional	No	Neurological assessment

Renaud et al.[89] (2011)	Clinical characteristics	Descriptive, proportion of children with EV-CNS-infection and PeV-CNS-infection	Selection of body specimens	Clinical evidence for CNS-infection, PeV positive CSF samples, and review of medical records	Age at onset of > 90 days	11/12 (92%)	Only medium-term follow-up of child with sequelae	Cross-sectional	No	Neurological assessment
Sano et al.[90] (2018)	Prevalence	Descriptive, proportion of children with EV-CNS-infection and PeV-CNS-infection	Based on reviewing of medical records	Clinical evidence of CNS-infection and clinical information compiled from medical records	Age at onset of > 90 days, coinfection	Not reported	Not reported	Cross-sectional	No	Neurological assessment
Skram et al.[91] (2014)	Clinical characteristics	Descriptive, proportion of children with PeV-CNS-infection	Based on reviewing of medical records and laboratory reports	Clinical evidence of CNS-infection and during hospitalisation or within a few weeks after discharge in a defined retrospective period	Age at onset of > 90 days and gestational age of < 37 weeks	14/15 (93%)	Only long-term follow-up of child with sequelae	Cross-sectional	No	Neurological assessment
Tang et al.[92] (2016)	Detection methods	Descriptive, proportion of children with PeV-CNS-infection	Consecutive	Clinical evidence for CNS-infection and hospitalisation	Coinfection	Not reported	Not reported	Cross-sectional	No	Not reported
Verboon-Maciolek et al.[7] (2008)	Outcome	Descriptive, proportion of children with PeV-CNS-infection	Consecutive, during hospitalisation	Clinical evidence for CNS-infection, hospitalisation, and retrospective inclusion of children hospitalised in a specific period	Age at onset of > 90 days and coinfection	1/10 (10%)	Only long-term follow-up of children with sequelae	Cross-sectional	No	Neurological assessment, standard visual functions screening, and GMDS

Table 2.2 continues on next page.

Table 2.2: ***Continued***

			Selection					Ascertainment of outcome		
Author (year)	Study objective*	Reporting	Recruitment of children	Inclusion criteria	Exclusion criteria	Lost to follow-up, *n/N* (%)	Reasons lost to follow-up	Data collection	Investigators masked for diagnosis outcome assessor	Standardised measurements
Verboon-Maciolek et al.[93] (2008)	Outcome	Descriptive, proportion of children with EV-CNS-infection and PeV-CNS-infection	Consecutive, during hospitalisation	Clinical evidence for CNS-infection and hospitalisation and presentation at emergency or outpatient departments	Age at onset of > 90 days and coinfection	Not reported	Not reported	Cross-sectional	No	GMDS
Vergnano et al.[94] (2015)	Outcome	Descriptive, proportion of children with PeV-CNS-infection	Based on reviewing of medical records	Clinical evidence for CNS-infection and hospitalised in a defined retrospective period	Age at onset of > 90 days	31/50 (62%)	Not reported	Cross-sectional	No	Neurological assessment and standard visual functions screening

AIMS: Alberta infant motor scale; ASQ-3: Ages and Stages questionnaire version 3; BSID-III: Bayley scales of infant and toddler development third edition (USA); Bayley-3-NL: Bayley scales of lifant and toddler development third edition Netherlands; CSF: cerebrospinal fluid; EV: enterovirus; GMDS: Griffith mental developmental scale; GOS: Glasgow outcome scale; PeV: human parechovirus; LOS-f/up: Liverpool outcome score-follow-up, 2013; M-ABC-2 NL: movement assessment battery for children version-2 Netherlands. * Study objective as part of our study aim.

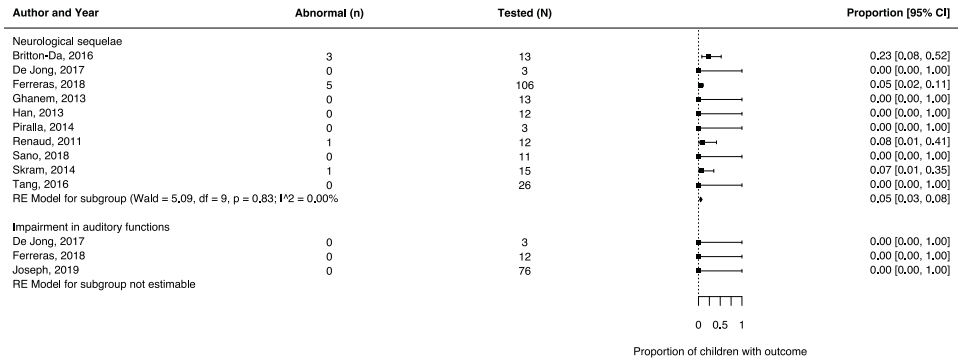

Figure 2.2: Neurological and neurodevelopmental outcomes at short-term follow-up.
Abbreviations: *df*: degrees of freedom; RE: random effects.

The results of eight studies (Figure 2.3) in the medium-term follow-up category could not be pooled in a meta-analysis because of high statistical heterogeneity (I^2 = 90.81% for neurological outcomes[7,17,81,82,86,87,89] and I^2 = 89.91% for gross motor function delay).[85,86] The only study that assessed speech and language function reported that one child of 77 had and abnormality.[86] None of the studies assessed auditory or visual function.

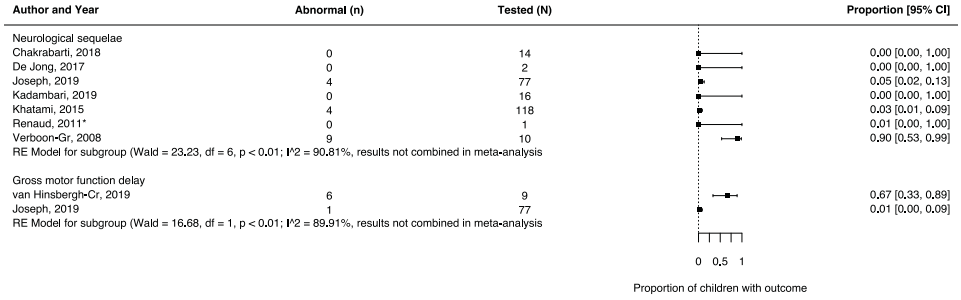

Figure 2.3: Neurological and neurodevelopmental outcomes at medium-term follow-up.
Abbreviations: *df*: degrees of freedom; RE: random effects. * The proportion in plot differs from 0 because of continuity correction.

Seven studies[7,79,80,82,86,93,94] in the long-term follow-up category assessed the outcomes of interest (Figure 2.4) and found neurological sequelae in between 0%[82] and 47% of children.[94] Pooled results showed that 27% developed neurological sequelae (pooled proportion 0.27 [95% CI 0.17–0.40], I^2 = 52.74%; p = 0.026). Four studies investigated visual problems,[7,79,86,94] and reported abnormalities in between 4%[86] and 36%[79] of children (pooled proportion

0.09 [0.03–0.25], I^2 = 57.81%; p = 0.017). Sensitivity analysis using a fixed-effect model showed similar results (pooled proportion 0.08 [0.04–0.14]). Gross motor function delay was reported in between 0%[82,91] and 45% of children.[79] Pooled results showed 19% with suspect gross motor function delay (pooled proportion 0.19 [0.08–0.39], I^2 = 64.69%; p = 0.012)[16,79,80,82,86,91] None of the studies in the long-term follow-up category investigated auditory functions.

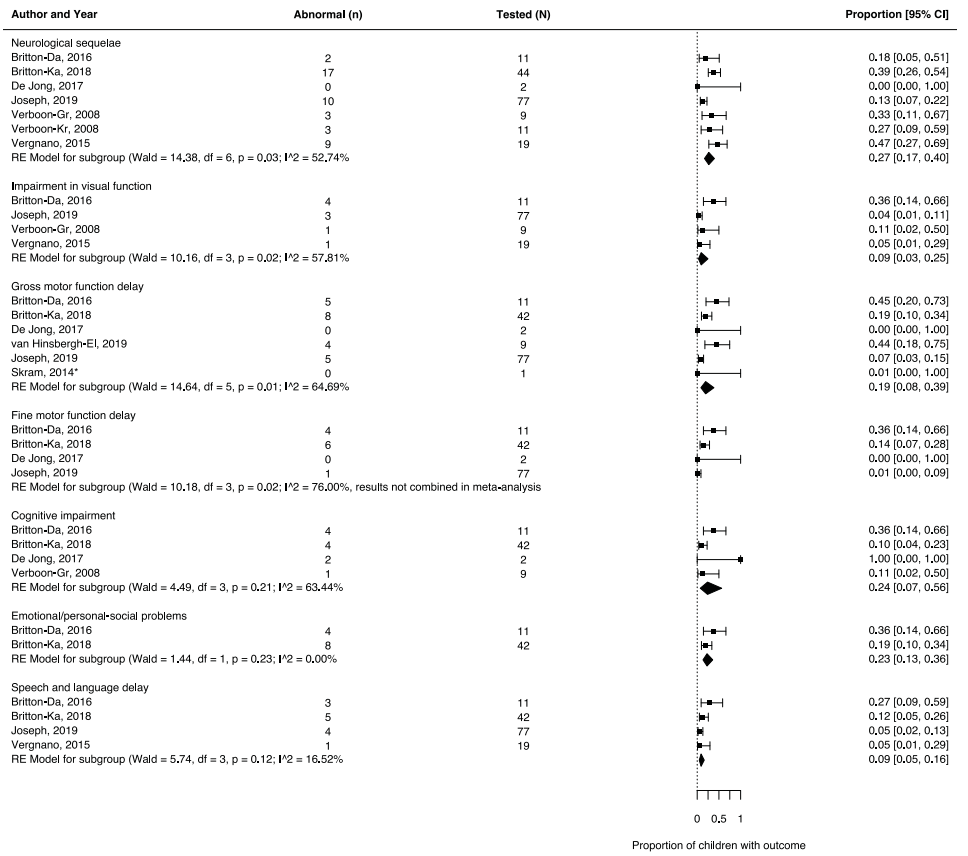

Figure 2.4: Neurological and neurodevelopmental outcomes at long-term follow-up.
Abbreviations: *df*: degrees of freedom; RE: random effects. * The proportion in plot differs from 0 because of continuity correction.

Studies that investigated secondary outcome variables showed suspect fine motor function delay in between 0%[82] and 36%[79] of children. These results were not pooled in a meta-analysis because of high statistical heterogeneity (I^2 = 76.00%). Cognitive impairment was reported in between 10%[80] and 100%[82] (pooled proportion 0.24 [95% CI 0.07–0.56], I^2 =

63.44%; $p = 0.21$),[7,79,80,82] emotional and personal-social problems in between 19%[80] and 36%[79] (pooled proportion 0.23 [0.13–0.36], $I^2 = 0.00\%$, $p = 0.23$), and speech and language development delay[79,80,86,94] in between 5%[86,94] and 27%[79] (pooled proportion 0.09 [0.05–0.16], $I^2 = 16.52\%$; $p = 0.12$). Sensitivity analyses using fixed-effect models yielded similar results for emotional and social problems (pooled proportion 0.23 [0.13–0.36]) and speech and language developmental delay (0.09 [0.05–0.14]). Results for cognitive impairment differed slightly (0.17 [0.10–0.28]). No study in this category investigated behavioural problems. In the meta-analyses we observed overlap of cohort participants in some studies at different follow-up timepoints. To investigate whether this observation had any effect on the pooled outcomes, we did post-hoc sensitivity analyses by excluding studies that reported on the same underlying cohorts from the meta-analyses.[6,79,80,92] Pooled results of either neurological sequelae at short-term or neurodevelopmental delay at long-term follow-up remained unchanged (data not shown).

Discussion

In this systematic review, we summarised and critically appraised studies on the neurological and neurodevelopmental outcomes of young children after parechovirus infection of the CNS. To our knowledge, this is the first meta-analysis of the parechovirus infection of the CNS. Overall, our findings indicate that follow-up is a substantial issue for young children with parechovirus infection of the CNS, a vulnerable population at risk for neurological sequelae and neurodevelopmental delay. They highlight the need for long-term follow-up, because subtle neurodevelopmental delay might manifest only after a year.

The meta-analyses showed an increasing average proportion of children who developed neurological sequelae over time (5% at ≤ 6 weeks and 27% at ≥ 12 months). At 12 months or later, 9% of children had visual problems, 19% had suspected gross motor function delay, 24% has suspected cognitive impairment, 23% had emotional or personal-social problems, and 9% had speech and language delay.

A review[95] of viral meningitis, including parechoviruses, showed that neurotropic parechovirus-3 was the genotype more associated with encephalitis than meningitis.[7,46] Most children with symptomatic parechovirus infection of the CNS did not have cerebrospinal fluid pleocytosis.[48,81] The clinical spectrum overlaps with those of other pathogens in neonates and young children. In real-life settings, clinical symptoms overlap; therefore, we included all spectra of parechovirus infection of the CNS (meningitis, encephalitis, meningoencephalitis, and sepsis-

like illness) in our systematic review. We reasoned that this approach would give a more complete clinical picture of the neurological sequelae and neurodevelopmental delay associated with parechovirus infection. It might explain the higher proportion of poor neurodevelopmental function reported in this study. We also investigated abnormalities in other important neurodevelopmental domains that might only manifest with time.[20,21] Our findings in these domains contribute to new knowledge in this field. Often, these late-onset abnormalities are accidentally discovered by parents or paediatricians, making it extremely difficult to relate them to parechovirus infection in early childhood (children < 3 months of age).

There is a high parechovirus-3 incidence in neonatal infections.[96] This genotype has previously been described as more neurotropic than other types.[7,46] The relatively high frequency of neurological sequelae and neurodevelopmental delay associated with parechovirus-3 might be a function of age of children. This might be because of age-related vulnerability of the developing brain to ischaemic and inflammatory damage in neonates and young children.[11,97] This theory is supported by seven studies in this Article, which included infants who were born preterm;[6,7,79,80,86,93,94] others did not specify the gestational ages of included children.[17,81,83,84,87,89,90,92] Neonates and young children not only often have the highest burden of parechovirus infection of the CNS, but they also have the most severe clinical manifestations often resulting in intensive care unit hospitalisation and the poorest outcomes.[6-9,11,12-15,17,79,80,86-88,93,94] In addition to age-related vulnerability of the developing white matter for ischaemic and inflammatory damage,[11,97] other factors thought to play a part in the vulnerability of neonates and young children to parechovirus infection of the CNS are their immature immune system and insufficient protective maternal antibodies.[98] However, there is scarce supportive evidence. Studies have shown periventricular white matter damage after MRI imaging of infants with parechovirus infection,[6-10,79,80,86,87,91,93,94] supporting the aforementioned vulnerability of the young brain to parechovirus infection. Only four studies reported children with MRI-confirmed encephalitis or meningoencephalitis.[7,79,93,94] Others reported only a small percentage of children with MRI-confirmed encephalitis or meningoencephalitis.[6,80,86,87,91] Although it is logical to assume that children with MRI-confirmed encephalitis (or meningoencephalitis) might have a worse outcome than those with only meningitis, the studies in this systematic review were not sufficiently powered to comparatively evaluate this assumption.

Australian studies that formed a substantial percentage of included studies reported worse average outcomes than those from Europe. This finding suggests the possible role of differences in geographical location or virulence of parechovirus strains.[99]

Our results showed a subtle neurodevelopmental delay in gross motor function, fine motor function, cognition, emotional and personal-social development, and speech and language at long-term follow-up. This result is in support of previous studies with long follow-up durations.[20-22] Early interventions might have a positive effect on these outcomes.[19,23,100] Neurological sequelae and neurodevelopmental delay in young children evolves with time because of the plasticity of the young brain.[11,100,101] Longitudinal cohort studies support this.[16,102]

A limitation of this systematic review is that we applied a cutoff of less than 1.0 *SD* from the age-adjusted norm for outcome measures to categorise children as abnormal. This cutoff might have resulted in an overestimation of the proportion of children with suspected neurodevelopmental delay. Although not all the children in this category might have had clinically substantial neurodevelopmental delay, identifying them might be practically relevant for periodic monitoring, to detect the first signs of neurodevelopmental delay. Early detection is important for initiating early intervention programmes to prevent permanent delay in their neurodevelopmental function, which is the reason we used the term delay instead of impairment.

A limitation of the included studies is the high level of heterogeneity between the source populations, designs, definitions of parechovirus infection of the CNS, outcomes of interest, selection methods, number of children followed up, duration of follow-up, and assessment methods for outcomes of interest. Some studies restricted participation to children no more than 90 days old at onset of infection, but others included older children. Some studies excluded children born preterm, a known independent risk factor for neurological sequelae and neurodevelopmental delay,[103,104] but others did not. Not all studies corrected for the influence of intensive care unit hospitalisation on short-term, medium-term, and long-term neurological and neurodevelopmental outcomes.

Most studies presented only one or two groups side by side for which we extracted only the cases with parechovirus infection of the CNS. This limited the meta-analyses to only the analysis of proportions and made adjustment of confounders impossible. Because few studies included control groups, we were unable to compare groups with parechovirus infection of the CNS with control groups. Previously, our research group assessed neurodevelopmental outcomes between children with parechovirus infection of the CNS compared with two control groups (with parechovirus infection elsewhere and with no pathogen detected). Some children in the control groups also fell into the test category below norm.[16,85] We used the tool proposed by Murad and colleagues[30] to assess the risk of bias. The tool provides

domains and explanatory questions to evaluate methodological quality; however, it does not provide clear guidance on judging domains at low risk, unclear risk, or high risk of bias.[30] Although we described the methodological quality of the included studies, to what extent bias affects the outcomes of our meta-analyses remains difficult to judge.

The aforementioned high heterogeneity between studies, methodological issues related to selection of children, ascertainment of diagnosis and outcomes, and forest plots of subgroups within fewer than four studies in the meta-analysis means that these results should be interpreted with caution.

In conclusion, the results of this systematic review and meta-analysis showed an increasing proportion of children with neurological sequelae and neurodevelopmental delay in time after parechovirus infection of the CNS. Most of these abnormalities were not clinically severe. However, because abnormalities in some domains might manifest much later, we recommend long follow-up of these children, preferably up to entry into school (age 5–6 years).

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Appendix 2.1: Search strategies

Search strategy for PubMed (18 March 2019)

Search	Query	Items found
#6	#1 AND #2 AND (#3 OR #4) AND #5	134
#5	"Meningitis"[Mesh:NoExp] OR "Meningitis, Viral"[Mesh] OR "Meningoencephalitis"[Mesh] OR "Meningitis, Aseptic"[Mesh] OR arachnoiditi*[tiab] OR cerebriti*[tiab] OR cerebral cryptococcos*[tiab] OR toruloma*[tiab] OR choriomeningiti*[tiab] OR armstrong syndrome[tiab] OR armstrong's syndrome[tiab] OR meningoencephaliti*[tiab] OR cerebromeningiti*[tiab] OR meningoencephaliti*[tiab] OR cerebriti*[tiab] OR mening*[tiab] OR pachymening*[tiab] OR intracranial[tiab] OR "central nervous system"[tiab] OR "Central Nervous System"[Mesh] OR "Central Nervous System Diseases"[Mesh] OR white matter[tiab] OR grey matter[tiab] OR gray matter[tiab] OR brain[tiab] OR encephal*[tiab] OR brainstem*[tiab] OR spinal[tiab] OR spine[tiab] OR arachnoid*[tiab] OR dura mater[tiab] OR pia mater[tiab] OR cerebr*[tiab] OR midbrain*[tiab] OR hydrocephal*[tiab] OR intracrani*[tiab] OR intercrani*[tiab] OR myeliti*[tiab] OR myelum[tiab] OR perimening*[tiab]	3,001,235
#4	"Epidemiologic Studies"[Mesh] OR cohort[tiab] OR (case[tiab] AND (control[tiab] OR controll*[tiab] OR comparison[tiab] OR referent[tiab])) OR risk[tiab] OR causation[tiab] OR causal[tiab] OR "odds ratio"[tiab] OR etiol*[tiab] OR aetiol*[tiab] OR "natural history"[tiab] OR predict*[tiab] OR prognos*[tiab] OR outcome[tiab] OR course[tiab] OR retrospect*[tiab] OR follow up[tiab] OR followup[tiab]	6,300,813
#3	(((review*[tiab] OR search*[tiab] OR survey*[tiab] OR handsearch*[tiab] OR hand-search*[tiab]) AND (databa*[tiab] OR data-ba*[tiab] OR bibliograph*[tiab] OR electronic*[tiab] OR medline*[tiab] OR pubmed*[tiab] OR embase*[tiab] OR Cochrane[tiab] OR cinahl[tiab] OR psycinfo[tiab] OR psychinfo[tiab] OR cinhal[tiab] OR "web of science"[tiab] OR "web of knowledge"[tiab] OR ebsco[tiab] OR ovid[tiab] OR mrct[tiab] OR metaregist*[tiab] OR meta-regist*[tiab] OR ((predetermined[tiab] OR pre-determined[tiab]) AND criteri*[tiab]) OR apprais*[tiab] OR inclusion criteri*[tiab] OR exclusion criteri*[tiab])) OR (review[pt] AND systemat*[tiab]) OR "systematic review"[tiab] OR "systematic literature"[tiab] OR "integrative review"[tiab] OR "integrative literature"[tiab] OR "evidence-based review"[tiab] OR "evidence-based overview"[tiab] OR "evidence-based literature"[tiab] OR "evidence-based survey"[tiab] OR "literature search"[tiab] OR ((systemat*[ti] OR evidence-based[ti]) AND (review*[ti] OR literature[ti] OR overview[ti] OR survey[ti])) OR "data synthesis"[tiab] OR "evidence synthesis"[tiab] OR "data extraction"[tiab] OR "data source"[tiab] OR "data sources"[tiab] OR "study selection"[tiab] OR "methodological quality"[tiab] OR "methodologic quality"[tiab] OR cochrane database syst rev[ta] OR meta-analy*[tiab] OR metaanaly*[tiab] OR metanaly*[tiab] OR meta-analysis[pt] OR meta-synthesis[tiab] OR metasynthesis[tiab] OR meta-study[tiab] OR metastudy[tiab] OR metaethnograph*[tiab] OR meta-ethnograph*[tiab] OR Technology Assessment, Biomedical[mh] OR hta[tiab] OR health technol assess [ta] OR evid rep technol assess summ[ta]	520,342

Search	Query	Items found
	OR health technology assessment[tiab]) OR ((review*[ot] OR search*[ot] OR survey*[ot] OR handsearch*[ot] OR hand-search*[ot]) AND (databa*[ot] OR data-ba*[ot] OR bibliograph*[ot] OR electronic*[ot] OR medline*[ot] OR pubmed*[ot] OR embase*[ot] OR cochrane[ot] OR cinahl[ot] OR psycinfo[ot] OR psychinfo[ot] OR cinhal[ot] OR "web of science"[ot] OR "web of knowledge"[ot] OR ebsco[ot] OR ovid[ot] OR mrct[ot] OR metaregist*[ot] OR meta-regist*[ot] OR ((predetermined[ot] OR pre-determined[ot]) AND criteri*[ot]) OR apprais*[ot] OR inclusion criteri*[ot] OR exclusion criteri*[ot]) OR (review[pt] AND systemat*[ot]) OR "systematic review"[ot] OR "systematic literature"[ot] OR "integrative review"[ot] OR "integrative literature"[ot] OR "evidence-based review"[ot] OR "evidence-based overview"[ot] OR "evidence-based literature"[ot] OR "evidence-based survey"[ot] OR "literature search"[ot] OR ((systemat*[ti] OR evidence-based[ti]) AND (review*[ti] OR literature[ti] OR overview[ti] OR survey[ti])) OR "data synthesis"[ot] OR "evidence synthesis"[ot] OR "data extraction"[ot] OR "data source"[ot] OR "data sources"[ot] OR "study selection"[ot] OR "methodological quality"[ot] OR "methodologic quality"[ot] OR meta-analy*[ot] OR metaanaly*[ot] OR metanaly*[ot] OR meta-analysis[pt] OR meta-synthesis[ot] OR metasynthesis[ot] OR meta-study[ot] OR metastudy[ot] OR metaethnograph*[ot] OR meta-ethnograph*[ot] OR hta[ot] OR health technology assessment[ot]))	
#2	infan*[tw] OR child*[tw] OR adolescen*[tw] OR pediatric*[tw] OR paediatric*[tw] OR pube*[tw] OR juvenil*[tw] OR school*[tw] OR newborn*[tiab] OR new-born*[tiab] OR neo-nat*[tiab] OR neonat*[tiab] OR premature*[tiab] OR postmature*[tiab] OR pre-mature*[tiab] OR post-mature*[tiab] OR preterm*[tiab] OR pre-term*[tiab] OR baby[tiab] OR babies[tiab] OR toddler*[tiab] OR youngster*[tiab] OR preschool*[tiab] OR kindergart*[tiab] OR kid[tiab] OR kids[tiab] OR playgroup*[tiab] OR play-group*[tiab] OR playschool*[tiab] OR prepube*[tiab] OR preadolescen*[tiab] OR junior high*[tiab] OR highschool*[tiab] OR senior high[tiab] OR young people*[tiab] OR minors[tiab]	4,284,981
#1	"Picornaviridae"[mesh:noexp] OR "Parechovirus"[mesh] OR parechovir*[tiab] OR "parecho virus"[tiab] OR picornavir*[tiab] OR sapelovir*[tiab] OR senecavir*[tiab] OR sicinivir*[tiab] OR tremovir*[tiab]	4,788

[Mesh]: Medical subject headings; [Mesh:NoExp]: MeSH without explosion; [tiab]: words in title OR abstract.

Search strategy for Embase.com (18 March 2019)

Search	Query	Items found
#6	#1 AND #2 AND (#3 OR #4) AND #5	273
#5	'meningitis'/de OR 'arachnoiditis'/exp OR 'aseptic meningitis'/exp OR 'meningoencephalitis'/exp OR 'subdural empyema'/exp OR 'virus meningitis'/exp OR 'central nervous system'/exp OR 'central nervous system disease'/exp OR arachnoiditi*:ti,ab,kw OR 'cerebral cryptococcos*':ti,ab,kw OR toruloma*:ti,ab,kw OR choriomeningiti*:ti,ab,kw OR 'armstrong syndrome':ti,ab,kw OR 'armstrong s syndrome':ti,ab,kw OR cerebromeningiti*:ti,ab,kw OR meningoencephaliti*:ti,ab,kw OR cerebriti*:ti,ab,kw OR mening*:ti,ab,kw OR pachymening*:ti,ab,kw OR intracranial:ti,ab,kw OR 'central nervous system':ti,ab,kw OR 'white matter':ti,ab,kw OR 'grey matter':ti,ab,kw OR 'gray matter':ti,ab,kw OR brain:ti,ab,kw OR encephal*:ti,ab,kw OR brainstem*:ti,ab,kw OR spinal:ti,ab,kw OR spine:ti,ab,kw OR arachnoid*:ti,ab,kw OR 'dura mater':ti,ab,kw OR 'pia mater':ti,ab,kw OR cerebr*:ti,ab,kw OR midbrain*:ti,ab,kw OR hydrocephal*:ti,ab,kw OR intracrani*:ti,ab,kw OR intercrani*:ti,ab,kw OR myeliti*:ti,ab,kw OR myelum:ti,ab,kw OR perimening*:ti,ab,kw	4,410,507
#4	('meta analysis'/exp OR 'review'/exp OR ((meta NEAR/3 analy*):ab,ti,kw) OR metaanaly*:ab,ti,kw OR review*:ti,ab,kw OR overview*:ti,ab,kw OR ((synthes* NEAR/3 (literature* OR research* OR studies OR data)):ab,ti,kw) OR (pooled AND analys*:ab,ti) OR (((data NEAR/2 pool*):ab,ti,kw) AND studies:ab,ti) OR medline:ab,ti,kw OR medlars:ab,ti,kw OR embase:ab,ti,kw OR cinahl:ab,ti,kw OR scisearch:ab,ti,kw OR psychinfo:ab,ti,kw OR psycinfo:ab,ti,kw OR psychlit:ab,ti,kw OR psyclit:ab,ti,kw OR cinhal:ab,ti,kw OR cancerlit:ab,ti,kw OR cochrane:ab,ti,kw OR bids:ab,ti,kw OR pubmed:ab,ti,kw OR ovid:ab,ti,kw OR (((hand OR manual OR database* OR computer*) NEAR/2 search*):ab,ti,kw) OR ((electronic NEAR/2 (database* OR 'data base' OR 'data bases')):ab,ti,kw) OR bibliograph*:ab,kw OR 'relevant journals':ab,kw OR (((review* OR overview*) NEAR/10 (systematic* OR methodologic* OR quantitativ* OR research* OR literature* OR studies OR trial* OR effective*)):ab,kw)) NOT ((((retrospective* OR record* OR case* OR patient*) NEAR/2 review*):ab,ti,kw) OR (((patient* OR review*) NEAR/2 chart*):ab,ti)) NOT ('editorial'/exp OR 'erratum'/de OR 'letter'/exp)	3,723,739
#3	cohort:ab,ti,kw OR (case:ab,ti,kw AND (control:ab,ti,kw OR controll*:ab,ti,kw OR comparison:ab,ti,kw OR referent:ab,ti)) OR risk:ab,ti,kw OR causation:ab,ti,kw OR causal:ab,ti,kw OR 'odds ratio':ab,ti,kw OR etiol*:ab,ti,kw OR aetiol*:ab,ti,kw OR 'natural history':ab,ti,kw OR predict*:ab,ti,kw OR prognos*:ab,ti,kw OR outcome:ab,ti,kw OR course:ab,ti,kw OR retrospect*:ab,ti,kw OR 'epidemiology'/de OR 'follow up':ab,ti,kw OR followup:ab,ti,kw	7,796,784
#2	adolescen*:ab,ti,kw OR 'adolescence'/exp OR 'adolescent coping orientation for problem experiences'/exp OR 'adolescent development'/exp OR 'adolescent disease'/exp OR 'adolescent health'/exp OR 'adolescent parent'/exp OR 'adolescent pregnancy'/exp OR 'adolescent smoking'/exp OR 'adolescent'/exp OR 'adolescent-family inventory of life events and changes'/exp OR babies:ab,ti,kw OR baby:ab,ti,kw OR 'birth weight'/exp OR boy:ab,ti,kw OR boyhood:ab,ti,kw OR boys:ab,ti,kw OR 'brazelton neonatal	5,356,465

Search	Query	Items found
	behavioral assessment scale'/exp OR 'child abuse'/exp OR 'child advocacy'/exp OR 'child behavior checklist'/exp OR 'child behavior'/exp OR 'child care'/exp OR 'child death'/exp OR 'child health care'/exp OR 'child health'/exp OR 'child nutrition'/exp OR 'child parent relation'/exp OR 'child psychology'/exp OR 'child restraint system'/exp OR 'child safety'/exp OR 'child welfare'/exp OR child*:ab,ti,kw OR 'child'/exp OR 'childhood disease'/exp OR 'childhood mortality'/exp OR 'childhood'/exp OR girl:ab,ti,kw OR girlhood:ab,ti,kw OR girls:ab,ti,kw OR 'high risk infant'/exp OR infan*:ab,ti,kw OR 'infant disease'/exp OR 'infant mortality'/exp OR 'infant nutrition'/exp OR 'infant welfare'/exp OR 'infanticide'/exp OR 'infantile diarrhea'/exp OR 'infantile hypotonia'/exp OR 'juvenile delinquency'/exp OR neonat*:ab,ti,kw OR 'neonatal weight loss'/exp OR 'newborn disease'/exp OR 'newborn morbidity'/exp OR 'newborn period'/exp OR newborn*:ab,ti,kw OR 'newborn'/exp OR nicu:ab,ti,kw OR 'only child'/exp OR paediatr*:ab,ti,kw OR pediatri*:de,ab,ti OR 'pediatric advanced life support'/exp OR 'pediatric anesthesia'/exp OR 'pediatric cardiology'/exp OR 'pediatric hospital'/exp OR 'pediatric intensive care nursing'/exp OR 'pediatric nurse practitioner'/exp OR 'pediatric nursing'/exp OR 'pediatric rehabilitation'/exp OR 'pediatric surgery'/exp OR 'newborn hypoxia'/exp OR 'pediatric ward'/exp OR 'pediatrics'/exp OR perinat*:ab,ti,kw OR 'perinatal development'/exp OR 'perinatal period'/exp OR 'persistent hyperinsulinemic hypoglycemia of infancy'/exp OR picu:ab,ti,kw OR postnat*:ab,ti,kw OR 'postnatal care'/exp OR 'postnatal development'/exp OR 'postnatal growth'/exp OR postneonat*:ab,ti,kw OR preschool*:ab,ti,kw OR puberty:ab,ti,kw OR 'runaway behavior'/exp OR 'school child':ab,ti,kw OR schoolchild*:ab,ti,kw OR 'severe myoclonic epilepsy in infancy'/exp OR suckling*:ab,ti,kw OR teen:ab,ti,kw OR teenager*:ab,ti,kw OR teens:ab,ti,kw OR toddler*:ab,ti,kw OR 'transient hypogammaglobulinemia of infancy'/exp OR youth:ab,ti,kw OR youths:ab,ti,kw	
#1	'picornaviridae'/de OR 'parechovirus'/exp OR 'tremovirus'/de OR 'sapelovirus'/exp OR parechovir*:ab,ti,kw OR 'parecho vir*':ab,ti,kw OR picornavir*:ab,ti,kw OR tremovir*:ab,ti,kw OR sapelovir*:ab,ti,kw OR senecavir*:ab,ti,kw OR sicinivir*:ab,ti,kw	6,394

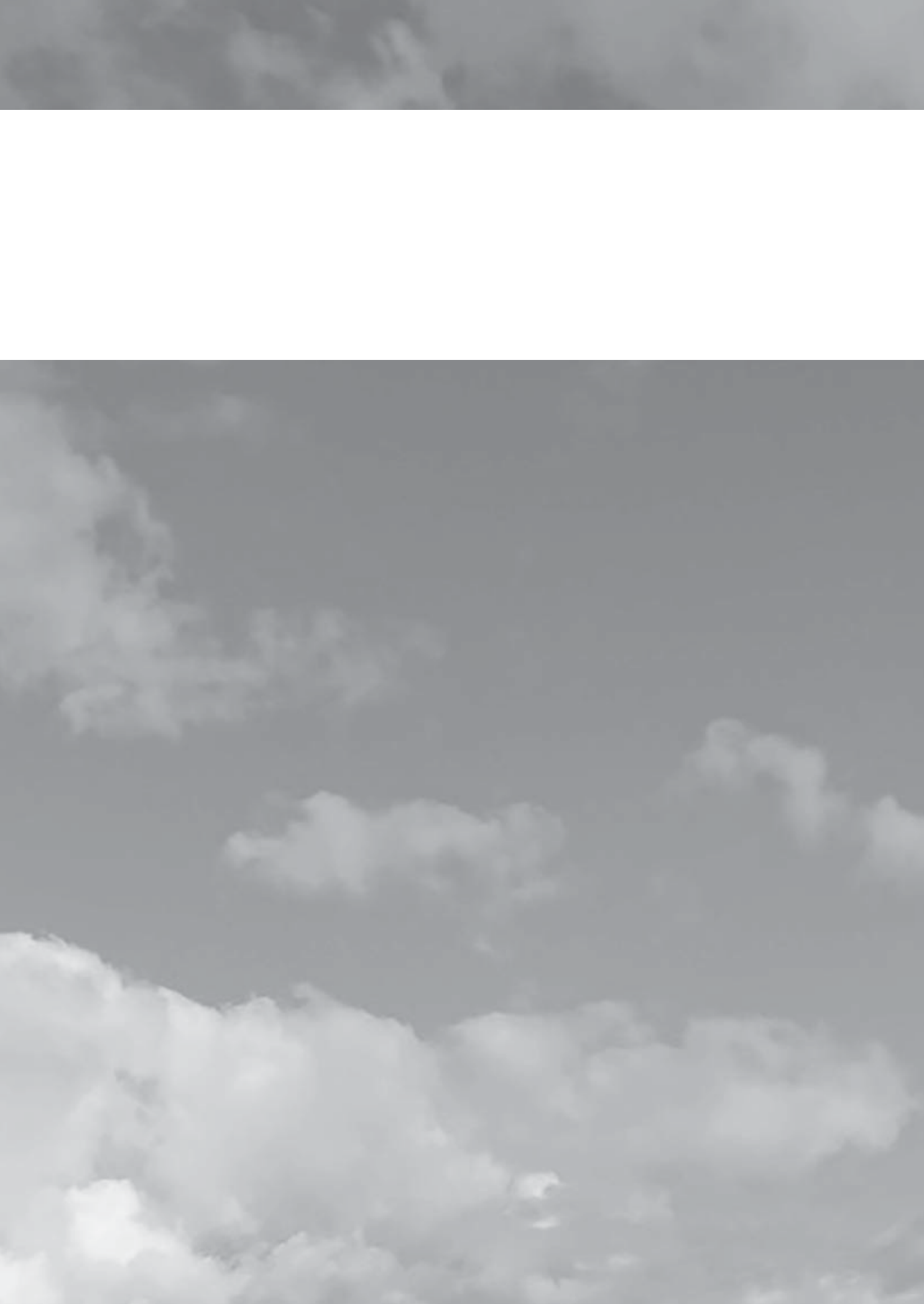
/exp: EMtree keyword with explosion; /de: EMtree keyword without explosion; :ti,ab,kw: words in title or abstract or author keywords.

Search strategy for Ebsco/PsycInfo (18 March 2019)

Search	Query	Limiters/expanders	Items found
S7	S5 OR S6	Search modes - Boolean/ Phrase	12
S6	S2 AND S4	Search modes - Boolean/ Phrase	8
S5	S2 AND S3	Search modes - Boolean/ Phrase	12
S4		Limiters - Age Groups: Childhood (birth-12 yrs), Neonatal (birth-1 mo), Infancy (2-23 mo), Preschool Age (2-5 yrs), School Age (6-12 yrs), Adolescence (13-17 yrs) Search modes - Boolean/ Phrase	750,722
S3	TI(adolescen* OR babies OR baby OR boy OR boyhood OR boys OR infan* OR neonat* OR newborn* OR nicu OR paediatr* OR pediatri* OR perinat* OR picu OR postnat* OR postneonat* OR preschool* OR puberty OR "school child" OR schoolchild* OR suckling* OR teen OR teenager* OR teens OR toddler* OR youth OR youths) OR AB(adolescen* OR babies OR baby OR boy OR boyhood OR boys OR infan* OR neonat* OR newborn* OR nicu OR paediatr* OR pediatri* OR perinat* OR picu OR postnat* OR postneonat* OR preschool* OR puberty OR "school child" OR schoolchild* OR suckling* OR teen OR teenager* OR teens OR toddler* OR youth OR youths) OR KW(adolescen* OR babies OR baby OR boy OR boyhood OR boys OR infan* OR neonat* OR newborn* OR nicu OR paediatr* OR pediatri* OR perinat* OR picu OR postnat* OR postneonat* OR preschool* OR puberty OR "school child" OR schoolchild* OR suckling* OR teen OR teenager* OR teens OR toddler* OR youth OR youths)	Search modes - Boolean/ Phrase	530,488
S2	TI(parechovir* OR 'parecho vir*' OR picornavir* OR tremovir* OR sapelovir* OR senecavir* OR sicinivir*) OR AB(parechovir* OR 'parecho vir*' OR picornavir* OR tremovir* OR sapelovir* OR senecavir* OR sicinivir*) OR KW(parechovir* OR 'parecho vir*' OR picornavir* OR tremovir* OR sapelovir* OR senecavir* OR sicinivir*)	Search modes - Boolean/ Phrase	32

Search	Query	Limiters/expanders	Items found
S1	TI("bornholm disease virus*" OR coxsackie* OR echovir* OR enterovir* OR "echo vir*" OR "entero vir*" OR "ljungan vir*" OR pasivir* OR "pleurodynia vir*" OR rosavir* OR hev OR hpev) OR AB("bornholm disease virus*" OR coxsackie* OR echovir* OR enterovir* OR "echo vir*" OR "entero vir*" OR "ljungan vir*" OR pasivir* OR "pleurodynia vir*" OR rosavir* OR hev OR hpev) OR KW("bornholm disease virus*" OR coxsackie* OR echovir* OR enterovir* OR "echo vir*" OR "entero vir*" OR "ljungan vir*" OR pasivir* OR "pleurodynia vir*" OR rosavir* OR hev OR hpev)	Search modes - Boolean/ Phrase	182

TI: words in title; AB: words in abstract; KW: words in author keywords.



Part 3

Cross-sectional assessment and assignment to subgroups

3

Human Parechovirus meningitis and gross motor neurodevelopment in young children

Ted M.T. van Hinsbergh
Stephanie C.M. de Crom
Robert Lindeboom
A. Marceline van Furth
Charlie C. Obihara

Eur J Pediatr. 2019;178:473-81

Abstract

This multicentre prospective cohort study describes the impact of human parechovirus meningitis on gross motor neurodevelopment of young children. Gross motor function was measured using Alberta infant motor scale. Of a total of 38 eligible children < 10 months of age at onset, 9 cases had clinical evidence of meningitis and Polymerase Chain Reaction positive for human parechovirus in cerebrospinal fluid, 11 had no meningitis and Polymerase Chain Reaction positive for human parechovirus in nasopharyngeal aspirate, blood, urine or faces and in 18 no pathogen was identified (reference-group).

The children with human parechovirus meningitis showed more frequent albeit not statistically significant suspect gross motor function delay (mean *Z*-score (standard deviation): -1.69 (1.05) than children with human parechovirus infection-elsewhere (-1.38 (1.51)). The reference-group did not fall in the range of suspect gross motor function delay (-0.96 (1.07)). Adjustment for age at onset and maternal education did not alter the results.

Conclusion: Six months after infection children with human parechovirus meningitis showed more frequent albeit not statistically significant suspect gross motor function delay compared to the population-norm and other two groups. Longitudinal studies in larger samples and longer follow-up periods are needed to confirm the impact and persistence of human parechovirus meningitis on neurodevelopment in young children.

Introduction

Human Parechovirus (HPeV) is progressively becoming a major viral cause of meningitis in children.[1-4] Especially HPeV-genotype 3 (HPeV-3) is an important cause of central nervous system (CNS) infection in young children,[3-5] HPeV-3 strains showed faster replication in neural cells and HPeV-3 receptor seems to facilitate entry of virus into neonatal CNS cells[6] and may cause both temporary and persistent white matter damage in the CNS,[7-11] leading to functional disorders as cerebral palsy (CP), developmental delay, tonus-regulation disorder, gross motor function (GMF) neurodevelopmental delay and retardation.[7,12-16]

There is a paucity of cohort studies that have prospectively evaluated short- and long-term motor and neurocognitive development of children with clinical evidence of meningitis and reverse transcriptase quantitative real-time polymerase chain reaction (RT-qPCR) positive for HPeV in cerebrospinal fluid (CSF).[7,13-17] So far, the follow-up studies that reported at least 3 children with HPeV-meningitis had focused primarily on neurological symptoms,[2,7,12,13,16] neuropsychological deficits using standardized neurodevelopmental scales[7] or standardized parental self-report-questionnaires.[12,16] To our knowledge none of these studies has yet performed a systematic GMF-assessment.[7,12,13] This is important since GMF-neurodevelopmental delay can be a good predictor of generalized developmental delay in young children. It is crucial to detect early signs of GMF-neurodevelopmental delay in order to refer children with delay for early intervention to maximize their development.[17,18]

The objective of this study was to prospectively investigate the GMF in a cohort of young Dutch children, 6 months after presenting with clinical evidence of meningitis and RT-qPCR positive for HPeV in CSF (HPeV CNS-infection) and to compare this with children from the same cohort with no clinical evidence of meningitis and RT-qPCR positive for HPeV in nasopharyngeal aspirate (NPA), blood, urine or faces (HPeV-infection-elsewhere) and those in whom no pathogens were identified (reference-group).

Methods

Summary of initial study

This study is part of a multicenter prospective cohort study conducted between March 2008 and September 2011 to investigate the incidence, clinical features, diagnostic methods and prognosis of HPeV- and enterovirus (EV)-infections in Dutch children. The study methods have been extensively described previously.[19,20] The study inclusion is shown in

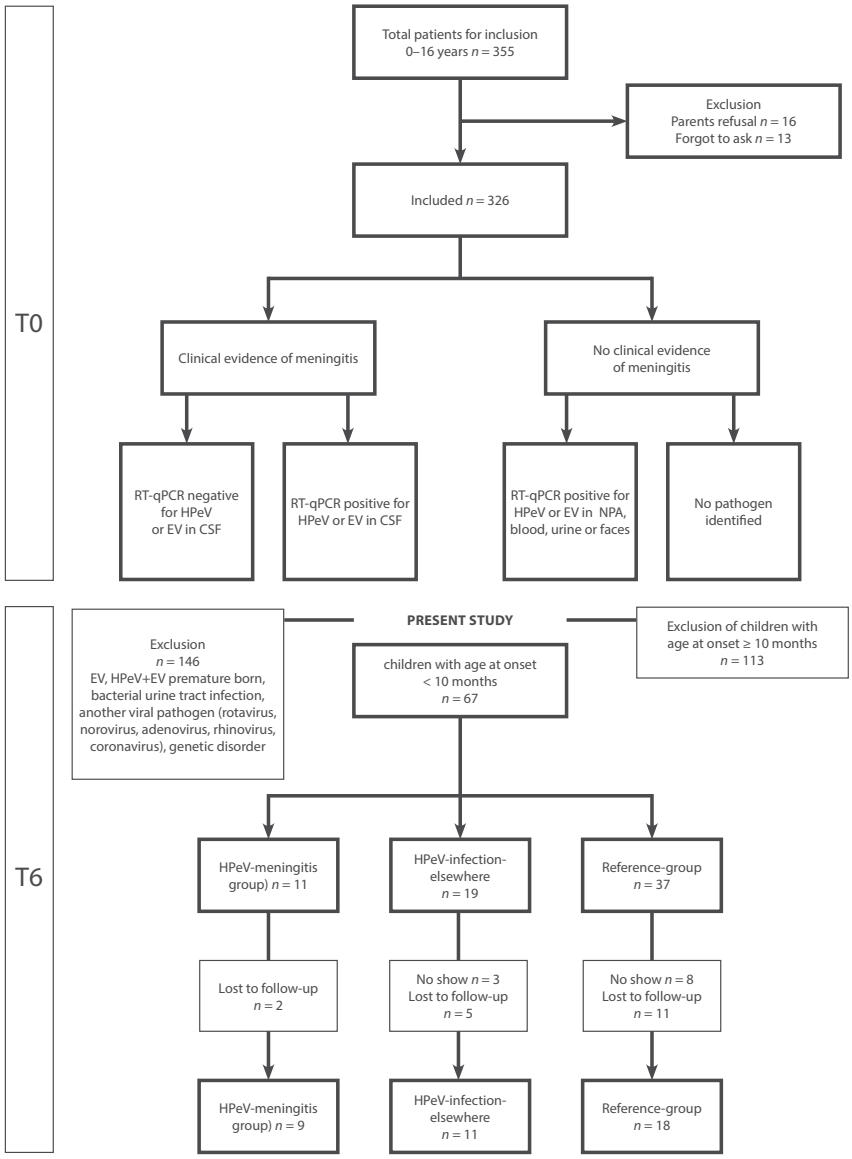


Figure 3.1: Flowchart inclusion of the children and follow-up 6 months after visiting the emergency or outpatient departments of the participating hospitals.
Abbreviations: CSF: cerebrospinal fluid; EV: enterovirus HPeV: human parechovirus; NPA: nasopharyngeal aspirate; n: number; RT-qPCR: reverse-transcriptase real time quantitative polymerase chain reaction; T0: presenting the emergency or outpatient departments of the participating hospitals; T6: follow-up 6 months after visiting the emergency or outpatient departments of the participating hospitals. Definitions: HPeV-meningitis: children with clinical evidence of meningitis and RT-qPCR positive for HPeV in CSF; HPeV-infection-elsewhere: children with no clinical evidence of meningitis and RT-qPCR HPeV-positive in nasopharyngeal aspirate swab, blood, urine or faces; Reference: children with no clinical evidence of meningitis and in whom no pathogen was identified; No show: did not show after 3x reminded; Lost to follow-up: moved to another address, changed telephone number, parents too busy to come.

the flowchart of Figure 3.1. Children 0–16 years with clinical suspicion of viral infection were eligible. Parents and/or legal guardians of eligible children received verbal and written information about the study and were invited to participate. Only those whose parents/legal guardians gave written informed consent were included. After inclusion the experienced consultant pediatrician on call at the hospital of presentation helped the patient and the parents complete a questionnaire on the birth and medical history, did physical examination and collected NPA, blood, urine and feces specimens for HPeV and EV RT-qPCR, feces and NPA specimens for viral culture.[19] If the experienced pediatrician clinically suspected the child to have meningitis or meningoencephalitis, a lumbar puncture was performed according to routine clinical practice and CSF specimen collected for chemistry, RT-qPCR for HPeV, EV and other common neurotropic viruses, including Herpes Simplex viruses 1 and 2, Varicella Zoster Virus and Adenovirus, viral culture and bacterial and fungal culture. Children with any viral pathogen than HPeV or any other bacterial or fungal pathogen and those with non-infectious explanation for their clinical picture, age at presentation > 16 years or non-consenting parents were excluded. Preterm-born children were also excluded because it is an important risk factor for GMF-neurodevelopmental delay, even in those without peri-and postnatal infection. The inclusion and exclusion criteria of the initial study are summarized in Table 3.1.

Assignment of study-children into subgroups

Based on medical history, presenting symptoms and finding on physical examination by the experienced pediatrician that saw the children at inclusion and the microbiology test-results, the participating children were assigned into 3 study-subgroups. The assignment took place after inclusion, 6 months prior to the present study to test the GMF of the younger children aged < 10 months at the initial study inclusion. The method of assignment during the initial study has been published elsewhere.[19] Children in whom their pediatrician found clinical evidence of meningitis[21] at presentation or thereafter during clinical observation and RT-qPCR positive for HPeV in CSF were assigned to the "HPeV-meningitis group"; those whose pediatrician found other clinical symptoms but no clinical evidence of meningitis at presentation or thereafter and RT-qPCR negative for HPeV in CSF, but RT-qPCR positive for HPeV in any of the other tested specimens (NPA, blood, urine or faces) were included in the "HPeV-infection-elsewhere group" and those in whom their pediatrician found other clinical symptoms but no clinical evidence of meningitis at presentation or thereafter and RT-qPCR negative for HPeV, EV and any of the other neurotropic viruses tested in CSF,

Table 3.1: Inclusion and exclusion criteria of the total study

Inclusion criteria
Children 0–16 year of age with at least one of the following clinical signs and symptoms:
1. Fever (temperature ≥ 38.0°C or ≥ 100.4°F)
or
2. Clinical evidence of meningitis including headache, photophobia, nuchal rigidity, irritability, lethargy, nausea, vomiting, drowsiness, positive sign of Kernig or Brudzinsky[a]
or
3. Other clinical signs and symptoms of infection: hypothermia, vomiting, diarrhea, anorexia, cough, myalgia, rash, hypovolemia or shock[b]
and
4. Signed informed consent by the parent(s)/legal guardian(s)
Exclusion criteria
1. Other proven infectious cause of the clinical symptoms
2. Other non-infectious cause of clinical symptoms: e.g. neoplasm, auto-immune diseases, rheumatic diseases, endocrinological diseases, gastro oesophageal reflux
3. Known severe psychomotor retardation, metabolic diseases with neuromuscular and/or cognitive abnormalities
4. Intra-uterine and perinatal problems or traumatic head injury
5. Preterm-born (gestational age < 37 weeks)
6. Children > 16 year of age

[a] At least 2 of these signs or symptoms must be present. [b] At least 3 of these signs or symptoms must be present.

feces, NPA, blood or urine specimens and negative viral, bacterial and fungal cultures were included in the "reference-group". The medical ethics committee of each participating center approved both study phases (NL-21361.008.07).

In the present follow-up sub study, we only invited eligible children aged < 10 months at the initial inclusion to participate. We sent an invitation letter to participate in this follow-up. In case of no response, we followed this up with telephone invitations on at least 2 occasions. If after the afore-mentioned attempts a parent could still not be reached we attempted to find out their forwarding addresses or telephone numbers via the practice of their GP in our hospital database. Prior to the visit parents/legal guardians were instructed not to reveal the study-subgroup to which their child was assigned during the initial study to the blinded independent pediatric physical therapist that tested their GMF-neurodevelopment.

During the follow-up, the GMF-neurodevelopment was tested in the pediatric outpatient departments of the participating hospitals by the same pediatric physical therapist. Before starting GMF-assessment each child was scored accordingly as alert, cooperative, actively moving, showing little activity, struggling and/or crying. By lack of cooperation

from the child, the GMF-assessment was rescheduled on another day. Each child's body mass, length and head circumference were recorded. Parents/legal guardians were asked to complete a questionnaire on their child's general health, use of medication, presence of recent traumatic head injury and early intervention (particularly pre-speech- and GMF-training). According to Statistics Netherlands-criteria maternal education was classified as low (primary school or lower vocational education), middle (middle vocational education) and high (higher vocational education or university degree).

GMF

We assessed the GMF-neurodevelopment with a norm-referenced observational and performance-based instrument: the Alberta infant motor scale (AIMS).[22] The AIMS,[22] originally developed to assess GMF-neurodevelopment in Canadian children, from birth to independent walking, has been shown to be discriminative and valid for cross-cultural use.[23] It is suitable for detecting minor GMF-neurodevelopmental delay in children aged 4–15 months.[23]

A higher raw score indicates a more mature GMF-neurodevelopment. The GMF raw scores were converted to age adjusted standard deviation (*SD*) scores (*Z*-score) for each age-month.[22] A *Z*-score of ≤ -1.30 (equivalent to the 10th percentile of the population norm (AIMS)) is considered suspect GMF-neurodevelopment delay,[23] while one *Z*-score difference is used to indicate a clinically relevant difference in GMF-neurodevelopment between groups.

Statistical analysis

The statistical analyses were performed with R-statistics (R-version 3.1.3, R Foundation for Statistical computing, Vienna, Austria). The analysis of categorical variables was performed with the Fisher exact test and continuous variables, including the *Z*-scores of the GMF-tests, with the one-way analysis of variance with post hoc Bonferroni correction and the Kruskal Wallis test with post hoc Mann-Whitney *U* test in case of non-normally distributed data. Linear regression was used to adjust for age at onset and maternal education. A *p*-value below 0.05 was considered to indicate statistical significance for all comparisons.

Results

Of the 213 children < 10 months at initial inclusion, 67 were eligible for participation in the follow-up study 6 months later and only 38 were included (Figure 3.1). Eighteen were lost-to-follow-up because they had moved elsewhere without any contact address or telephone number. Eleven failed to appear after repeated invitations; 146 were excluded for different reasons shown in Figure 3.1.

Study-population characteristics

Table 3.2 shows the baseline characteristics. Nine children (24%) had been assigned into the HPeV-meningitis group, 11 (29%) into the HPeV-infection-elsewhere group and 18 (47%) into the reference-group. There were no differences in baseline characteristics of the groups, except the age at onset. Children in the "HPeV-meningitis group" were younger than those in the other groups ($p = 0.01$) (Table 3.2). Of the 9 children in the HPeV-meningitis group, 8 (89%) were infected by HPeV-3. In 1 (11%) the HPeV-genotype was non-typeable. In the HPeV-infection-elsewhere group, 6 children (55%) were infected by HPeV-3, 3 (27%) by HPeV-1 and in the remaining 2 (18%) the HPeV-genotype was non-typeable. Of the most frequent presenting clinical symptoms, only irritability was significantly higher in the HPeV-meningitis group (Table 3.2).

Results of GMF-assessment, 6 months after presentation

At the follow-up 6 months after presenting at emergency or outpatient departments, parents complete a questionnaire. The groups did not differ in anthropometrical parameters, general health condition, medication use and presence of a recent traumatic head injury at the time of GMF-assessment. Seven children had positional nonsynostotical plagiocephaly, 1 in the HPeV-meningitis- and 2 in the HPeV-infection-elsewhere- and 4 in the reference-group. They all had a history of preferential supine position with asymmetric position of the head in bed.

Norm-referenced GMF of the study-children

Table 3.3 summarizes the norm-referenced GMF scores of the included children. Six of the 9 children (67%) in the HPeV-meningitis group had suspect GMF-neurodevelopmental delay (Z-score ≤ -1.30), compared with 45% in the HPeV-infection-elsewhere and 44% in the reference groups.

Table 3.2: Baseline characteristics of the included children attending follow-up visit

	HPeV-meningitis *n* = 9	HPeV-infection-elsewhere *n* = 11	Reference *n* = 18
HPeV-1	0 (0)	3 (27)	
HPeV-3	8 (89)	6 (55)	
HPeV non-typeable	1 (11)	2 (18)	
Male	8 (89)	6 (55)	9 (50)
Age at onset (days)			
Mean (*SD*)*	31.0 (17.2)	123.0 (73.4)	97.2 (90.5)
Min/max	13/68	16/275	14/251
Hospital stay (days)			
Mean (*SD*)	3.6 (0.7)	2.9 (1.6)	2.9 (2.2)
Min/max	3.0/5.0	0.0/4.0	0.0/8.0
Maternal education			
Low[1]	1 (11)	1 (9)	3 (17)
Middle[2]	5 (56)	5 (46)	3 (17)
High[3]	3 (33)	5 (45)	12 (66)
Clinical features			
Fever	9 (100)	10 (90.9)	16 (88.9)
Irritability*	7 (77.8)	6 (54.5)	5 (27.8)
Lethargy	2 (22.2)	4 (36.4)	4 (22.2)
Seizures	0 (0.0)	0 (0.0)	0 (0.0)
Dyspnea	1 (11.1)	2 (18.2)	1 (5.6)
Rash	3 (33.3)	6 (54.5)	5 (27.8)
Vomiting	1 (11.1)	4 (36.4)	6 (33.3)
Diarrhea	1 (11.1)	5 (45.5)	9 (50.0)
Poor feeding	5 (55.6)	6 (54.5)	8 (44.4)
CSF findings			
WBC (mm^{-3}) mean (*SD*)	5.7 (10.8)		
Min/max	1/30		
Protein (g/L) mean (*SD*)	0.45 ((0.11)		
Min/max	0.29/0.61		
Glucose (mmol/L) mean (*SD*)	2.98 (0.42)		
Min/max	2.60/3.60		
Pleocytosis	0 (100.0)		

* $p < 0.05$.

Numbers indicate absolute frequencies, relative frequencies between brackets unless otherwise indicated.

Abbreviations: *SD*: standard deviation of the mean; CSF: cerebrospinal fluid; HPeV: Human Parechovirus; min/max: minimum to maximum; n: number of children attending follow-up visit.

Education level of the parents: [1] Primary school and lower vocational education; [2] Middle vocational education; [3] Higher vocational education or university degree, according to the classification of Statistics Netherlands.

Definitions: Pleocytosis: the presence of elevated leukocyte count for age in the CSF. The age-specific reference values used were: CSF white blood cell (WBC) count > 22/μL for infants ≤ 4 weeks of age, > 15/μL for infants 4–6 weeks of age and > 7/μL for children > 6 weeks of age; HPeV-meningitis: children with clinical evidence of meningitis and RT-qPCR positive for HPeV in CSF; HPeV-infection-elsewhere: children with no clinical evidence of meningitis and RT-qPCR HPeV-positive in nasopharyngeal aspirate, blood, urine or faces; Reference: children with no clinical evidence of meningitis and in whom no pathogen was identified.

Table 3.3: Norm-referenced gross motor function neurodevelopment of the study-children 6 months after visiting the emergency or pediatric outpatient departments

	HPeV-meningitis *n* = 9	HPeV-infection-elsewhere *n* = 11	Reference *n* = 18
Children with GMF-neurodevelopmental delay	6/9 (67)	5/11 (45)	8/18 (44)
Mean GMF *Z*-score (SD)[a]	-1.69 (1.05)	-1.38 (1.51)	-0.96 (1.07)
Min/max	-3.14/-0.24	-4.56/0.25	-3.50/0.46

Total sample: children with age at onset 13 to 275 days. Number of children with GMF-neurodevelopmental delay / total number of children tested. Numbers indicate absolute frequencies, relative frequencies between brackets unless otherwise indicated. [a] Norm-referenced GMF *Z*-scores. *Z*-scores of ≤ -1.30 (equivalent to the 10th percentile of the population norm, measured with the Alberta infant motor scale) are generally accepted to indicate a suspect GMF-neurodevelopmental delay.
Abbreviations: *SD*: standard deviation; GMF: gross motor function; HPeV: Human Parechovirus; min/max: minimum to maximum; *n*: number of children attending follow-up visit. Definitions: HPeV-meningitis: children with clinical evidence of meningitis and RT-qPCR positive for HPeV in CSF; HPeV-infection-elsewhere: children with no clinical evidence of meningitis and RT-qPCR HPeV-positive in NPA, blood, urine or faces; Reference: children with no clinical evidence of meningitis and in whom no pathogen was identified.

Both HPeV-infection groups had a mean GMF *Z*-score in the range of a suspect GMF-neurodevelopmental delay. Children with HPeV-meningitis had notably, albeit not statistically significant lower mean GMF *Z*-scores (-1.69 versus -1.38 and 0.96 for the HPeV-infection-elsewhere and reference groups, respectively).

Association between HPeV-infection and GMF

Table 3.4 shows that, generally, children in the HPeV-meningitis group had lower GMF *Z*-scores compared to the children in the HPeV-infection-elsewhere group and in the reference group (regression coefficient beta (β) (95% Confidence Interval (CI): -0.74 (-1.74 to 0.26). Adjustment for age at onset and maternal education did not alter the results for the HPeV-meningitis group (β (95% CI) respectively: -0.72 (-1.81 to 0.36); -0.84 (-1.89 to 0.21)). Early intervention had no influence on the association HPeV-meningitis and GMF-neurodevelopment (β (95% CI): -0.74 (-1.71 to 0.23)).

Discussion

In this study we investigated the GMF-neurodevelopmental outcome of young Dutch children in the HPeV-meningitis group and compared this with those of children in the HPeV-infection-elsewhere group and those in the reference group, 6 months after presentation. A higher percentage of the children in the HPeV-meningitis group showed

Table 3.4: Association between human parechovirus meningitis and gross motor function neurodevelopment

Determinant	Unstandardized β[a] (95% CI)	*p*-value model[b]
Unadjusted model		0.31
HPeV-meningitis	-0.74 (-1.74 to 0.26)	
HPeV-infection-elsewhere	-0.43 (-1.37 to 0.51)	
Model adjusted for age at onset		0.51
HPeV-meningitis group	-0.72 (-1.81 to 0.36)	
HPeV-infection-elsewhere group	-0.43 (-1.39 to 0.53)	
Age at onset	-0.01 (-0.01 to 0.01)	
Model adjusted for maternal education		0.42
HPeV-meningitis	-0.84 (-1.89 to 0.21)	
HPeV-infection-elsewhere	-0.49 (-1.45 to 0.47)	
Maternal education high	-0.30 (-1.14 to 0.54)	
Model adjusted for early intervention		0.15
HPeV-meningitis	-0.74 (-1.71 to 0.23)	
HPeV-infection-elsewhere	-0.32 (-1.24 to 0.60)	
Early intervention	-0.77 (-1.67 to 0.11)	

[a] Unstandardized regression coefficient beta (β) indicates the differences in mean *Z*-score for HPeV-meningitis-group and HPeV-infection-elsewhere compared to children in the reference group (reference category). One *Z*-score difference is used to indicate a clinically relevant difference in GMF-neurodevelopment between groups. [b] Model *F*-statistic *p*-value.
Abbreviations: HPeV: Human Parechovirus; GMF: gross motor function; *SD*: Standard Deviation; min/max: minimum to maximum. Definitions: HPeV-meningitis: children with clinical evidence of meningitis and RT-qPCR positive for HPeV in CSF; HPeV-infection-elsewhere: children with no clinical evidence of meningitis and RT-qPCR HPeV-positive in NPA, blood, urine or faces; Reference: children with no clinical evidence of meningitis and in whom no pathogen was identified.

more frequent albeit not statistically significant suspect GMF-neurodevelopmental delay (*Z*-score of ≤ -1.30, equivalent to the 10th percentile of the population norm (AIMS-test)) than the children in the other two groups. Adjustment for age at onset, maternal education and early intervention did not alter the results. As with previous studies all the children in the HPeV-meningitis group were ≤ 3 months of age at inclusion and HPeV-3 was the most often isolated genotype.[2-4]

Our study-results are noteworthy in the context of and given support by a number of broader emerging literature in this area that reported variable neurodevelopmental outcomes, ranging from normal to CP in young children with HPeV-3-meningitis.[7,12-16] One of the hypotheses is that abnormalities in white matter seen during HPeV-infection may lead to minor and major motor functional delay.[7,12,13] In this study, children in the HPeV-meningitis group with suspect GMF-neurodevelopmental delay showed problems mainly in the

domain of postural control, a possible early sign for CP or Developmental Coordination Disorder (DCD).[24,25] These aren't enough follow-up data available to predict if some of these children may end up with CP or DCD at a later age. Barnett et al. have suggested the need for a long follow-up period of at least five years in children, in order to detect any late neurodevelopmental abnormalities.[26] The development of the human brain is a dynamic process and the nervous system changes continuously even after the first years of life.[27-29] The GMF-neurodevelopment might be variable over the course of the first years of life.[17,27] This means that GMF-neurodevelopmental delay, detected shortly after HPeV-meningitis may become reversible with time. Seven children, distributed between the 3 study-groups, presented with positional nonsynostotical plagiocephaly during the follow-up study. These were not caused by HPeV-infection but resulted from preferential supine positioning with asymmetrical position of the head in bed. The target of the early intervention was instruction on daily head positioning.

It is difficult to compare our results with the results of some previous findings with regards to severity.[7,12,13] This is mainly due to differences in gestational ages of participating children in the different studies. Follow-up studies that reported at least 3 children with HPeV-meningitis, such as those by Verboon et al. (n = 10), Britton et al. (n = 13) and Vergnano et al. (n = 19) all included a mixture of pre- and full-term-born children (born between 25–40 weeks).[7,12,13] It is known that preterm-born children are more likely to develop GMF-neurodevelopmental delay during the first 18 months of life, even in the absence of a HPeV-meningitis.[30] Therefore we excluded preterm-born children to limit its potential bias in our results. We recruited study-children from 3 general hospitals. They were generally mildly to moderately ill. None of them was admitted in an intensive care unit during the study. This contrasts with the afore-mentioned studies in which a majority of patients were admitted in a neonatal or pediatric intensive care unit.[7,12,13] Unlike those studies the children in our study are more representative of the general population of young Dutch children with HPeV-meningitis, presenting at emergency or outpatient departments.

Maternal education and other environmental factors may influence the GMF of young children.[7] There was no baseline difference in maternal education level between the 3 groups. This variable did not alter the effects of HPeV-meningitis on GMF in the multivariate regression analysis.

One of the strengths of this study is the use of 2 control groups, pooled from the same source-population of febrile children aged < 10 months, included in the initial study. Experienced pediatricians assigned the subjects to their study groups based on their history, clinical

symptoms, and physical examination and laboratory results in the way it is done in the daily clinical practice. Neither the blinded assessor of the GMFs nor the statistician that performed the statistical analyses of this study was involved in the initial group assignments. This is the first multicenter prospective study comparing GMF-neurodevelopment of children with proven HPeV-meningitis with 2 control groups of children with HPeV-infection-elsewhere and a reference-group with no pathogen isolated, respectively, from the same cohort. Despite the lack of difference in baseline characteristics, except for age at onset, the children in the HPeV-infection-elsewhere group also had a mean GMF *Z*-score in the range of a suspect GMF-neurodevelopmental delay. We cannot completely exclude the possibility of a child in the HPeV-meningitis group presenting without clinical evidence of meningitis at inclusion and being therefore misclassified and assigned into the HPeV-infection-elsewhere group. However, the fact that most children were hospitalized and clinically observed for an average of 3 days would make this unlikely. We did not consider it ethically acceptable to perform lumbar puncture in all children, irrespective of signs of clinical evidence of meningitis. We think it unlikely that children in the reference-group with meningitis were missed at inclusion, with no signs of clinical evidence of meningitis and negative RT-qPCR and culture for HPeV in all their body specimens. The decision not to perform neuro-imaging was based on pediatric literature that has shown discrepancies between brain MRI imaging and neurodevelopmental outcome variables.[7,12-16] Most of the reported brain abnormalities seen in the acute phase of infection either disappeared spontaneously or did not correlate to the GMF-neurodevelopment.

Among the strengths of this study is the use of a norm-referenced GMF-measurement (the AIMS). It is widely accepted for detecting (suspect) GMF-neurodevelopmental delays in young children. Information bias is unlikely because the pediatric physical therapist that conducted the GMF-test was blinded to the assigned study-groups of the children during the initial inclusion. The inclusion and exclusion criteria were strictly predefined and study-children were recruited consecutively, thereby reducing selection bias. A high proportion of children of the reference-group had GMF-neurodevelopmental delay. These children had severe symptoms that required a hospital visit. This may have influenced their GMF-neurodevelopment as well, and therefore induced the lack of difference in GMF-neurodevelopment we found between the HPeV-meningitis and reference groups. We carried out the GMF-testing after 6 months of infection to limit any effect of fever and hospitalization at the initial presentation on the GMF-neurodevelopment of the study-groups.

This study also has several limitations. Firstly, the number of children with HPeV-meningitis was low. This limitation is not specific for our study. Other prospective studies

conducted in Western countries have consistently found low incidences of HPeV-meningitis in children.[3,4,7,12,14-16] Secondly, as with many studies in neonates and young children it is impossible to adjust for all existing genetic, immunologic and environmental variables that could play a role in pregnancy, birth and perinatal development. We excluded those variables known to affect neurodevelopment, including preterm-birth, congenital and birth defects and infections. In the multivariate analyses-model we adjusted for the influence of age at onset of infection, maternal education level and early intervention. Children in the HPeV-meningitis group were younger than those in the other two groups. Adjustment for the age at onset did not alter the association between HPeV-meningitis and GMF-neurodevelopment (Table 3.4). Lastly, we limited our neurodevelopmental testing to the GMF because it is one of the earliest signs of generalized neurodevelopmental delay in young children. We did not measure language-, neuropsychological-, cognitive- and behavioral developments. With the plasticity of the young brain it is possible that GMF-neurodevelopment can still vary within the first years of life leading to late impairment.[28,29] We therefore recommend a longer period of longitudinal follow-up in similar cohorts of children until school-going age, with the addition testing of language-, neuropsychological-, cognitive- and behavioral skills. This will ascertain if a suspect GMF-neurodevelopmental delay is of a temporary or permanent nature and involvement of other parts of the brain.

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4

Motor neurodevelopment of children after a human Parechovirus or Enterovirus infection: 24 months follow-up

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Abstract

Introduction: Human Parechovirus (HPeV) and Enterovirus (EV) are known causes of viral infection and meningitis in childhood. Not much is known about the motor neurodevelopment of young Caucasian children after these infections. Most studies in the literature involved Asian children with only EV-71 infection, which is not prevalent in Western countries.

Methods: In this prospective multicenter blinded cohort study we tested the motor function level of children 24 months after an HPeV- or EV-infection (meningitis or elsewhere) and uninfected peers, with Bayley scales of infant and toddler development version-3 (BSID-III) and Movement assessment battery for children version-2 (M-ABC-2-NL). The total motor neurodevelopmental outcome, the gross motor function (GMF) neurodevelopmental outcome and the fine motor function (FMF) neurodevelopmental outcome were measured. Suspect motor neurodevelopmental delay (below the norm) was defined as a *Z*-score ≤ -1.

Results: Of the 157 analyzed children, the total motor neurodevelopmental outcome was below the norm in 16 (10%), the GMF-neurodevelopment was below the norm in 26 (27%) and FMF-neurodevelopment was below the norm in 9 (6%) children. There was no significant difference between the outcome of children with a meningitis, an infection elsewhere and uninfected peers.

Conclusion: No significant differences in motor neurodevelopment were found between HPeV- or EV-infected and uninfected Dutch children after 24 months of follow-up.

Introduction

Human Parechovirus (HPeV) and human Enteroviruses (EV) are frequent causes of viral infection and meningitis in children. Little is known about the motor neurodevelopment outcome of children after an HPeV/EV-infection. The only studies available in the literature on neurodevelopmental outcome after HPeV infection involved low powered studies in selected patient populations (< 2 months of age and mostly admitted to an intensive care unit (ICU).[1-4] Neurodevelopmental delay has been reported in Asian children with severe EV-71 central nervous system (CNS) infection.[5-9] There are, to our knowledge, no recent prospective cohort or case-control studies reporting on motor neurodevelopment outcome of non-Asian children after HPeV- and EV-infection. The purpose of this study is to determine the motor neurodevelopment outcome of children, 24 months after a HPeV- or EV-CNS-infection and to compare this with children with non-CNS-infection and peers in which no pathogen could be identified.

Methods

Study design

This study is part of a multicenter prospective cohort study to evaluate the incidence, clinical features and prognosis of HPeV- and EV-infection in children. We compared the motor neurodevelopment of the children 24 months after inclusion. Children were included in two of the largest non-university teaching hospitals (St. Elisabeth Hospital in Tilburg and Amphia Hospital in Breda) and in a non-teaching hospital (Tweesteden Hospital in Tilburg), the Netherlands, between 1st of March 2008 and 30th of September 2011.

Patients and enrolment

The study enrolment has been extensively described previously, and is shown in Table 4.1.[10,11] Nasopharyngeal, blood, urine and feces specimens were collected for HPeV and EV reverse-transcriptase real time quantitative polymerase chain reaction (RT-qPCR), and feces and nasopharyngeal specimens for viral culture. Children suspected to have a CNS-infection as judged by the treating pediatrician underwent a lumbar puncture and cerebrospinal fluid (CSF) specimen was collected for HPeV and EV RT-qPCR and bacterial and viral culture.

Table 4.1: Inclusion and exclusion criteria

Inclusion criteria
Children 0–16 year of age with at least one of the following clinical signs and symptoms: 1. Fever (temperature ≥ 38.0°C or ≥ 100.4°F) *or* 2. Clinical evidence of meningitis including headache, photophobia, nuchal rigidity, irritability, lethargy, nausea, vomiting, drowsiness, positive sign of Kernig or Brudzinsky[a] *or* 3. Other clinical signs and symptoms of infection: hypothermia, vomiting, diarrhea, anorexia, cough, myalgia, rash, hypovolemia or shock[b] *and* 4. Signed informed consent by the parent(s)/legal guardian(s)
Exclusion criteria
1. Other proven infectious cause of the clinical symptoms 2. Other non-infectious cause of clinical symptoms: e.g. neoplasm, auto-immune diseases, rheumatic diseases, endocrinological diseases, gastro oesophageal reflux 3. Known severe psychomotor retardation, metabolic diseases with neuromuscular and/or cognitive abnormalities 4. Intra-uterine and perinatal problems or traumatic head injury 5. Preterm-born (gestational age < 37 weeks) 6. Children > 16 year of age

[a] At least 2 of these signs or symptoms must be present. [b] At least 3 of these signs or symptoms must be present.

Detection methods

RT-qPCR

An aliquot (200 µl) of nasopharynx, blood, urine, feces or CSF specimen was used to extract (viral) RNA as described previously.[11,12] The isolated (viral) RNA was analyzed for the presence of HPeV and EV using an HPeV- and EV-specific RT-qPCR and used for genotyping as previously described.[13,14]

Viral culture

Viral culture was performed on confluent layers of tertiary Cynomolgus monkey kidney cells. After inoculation of 0.25 ml of clinical specimen and absorption in the cells for 1 hour, 1 ml of culture medium was added and cells, maintained at 37°C on roller drums, were examined daily during 14 days for a cytopathic effect. Genotyping of the virus isolates was carried out by neutralization or complement fixation with intersecting antiserum pools by standard procedures.

Follow-up

Included children were invited for a follow-up visit 24 months after inclusion. Motor neurodevelopment was assessed with the Bayley scales of infant and toddler development version-3 (BSID-III) and the Movement assessment battery for children version-2 (M-ABC-2-NL).[15,16] The pediatric physical therapist, who conducted the test in the children, was blinded to their earlier clinical diagnosis. In the invitation letter, parents and legal guardians were strictly instructed not to share the diagnosis of their child to the pediatric physical therapist.

BSID-III

The standardized BSID-III for Dutch children was used to determine the overall motor and mental development of children. This test is used for children aged between 16 days and 42.5 months.[15] The development can be assessed in five domains: cognition, language, motor, social-emotional and adaptive behavior. In this study, only the domain motor neurodevelopment of children was tested. This domain can be divided into two subscales, fine and gross motor skills. Since the BSID-III was not yet available in our hospital before we tested the first six children, they were tested with the BSID-II. The most important disadvantage of the BSID-II is that the subscale fine and gross motor skills are not split. The BSID-II contains other fine motor items than the BSID-III. Therefore, it was not possible to convert fine motor items from BSID-II to BSID-III. The advantage of the BSID-III is that it is possible to test the fine and gross motor skills separately.

M-ABC-2-NL

This test is a standardized discriminative instrument to detect motor neurodevelopmental problems in children between the ages of 42 months and 16 years. It is also standardized for Dutch children.[16] The test consists of eight test items, which are divided into three domains: manual dexterity, throw and catch, and static and dynamic balance.

Raw scores, standard scores for individual items, component standard scores with percentiles and total standard score with a corresponding percentile are provided with both tests. The total test-score can be divided in impairment in the motor neurodevelopment (i.e., score ≤ 5th percentile / Scale score < 5), a suspect motor neurodevelopmental delay (below the norm, i.e., score ≤ 16th percentile / Scale score 5–7) and a normal motor neurodevelopment (i.e., score > 16th percentile / Scale score ≥ 8). The total motor neurodevelopmental outcome can be divided into a GMF-neurodevelopmental and a FMF-neurodevelopmental outcome.

All the results of the motor function neurodevelopmental tests (BSID-II, BSID-III and M-ABC-2-NL) were converted to standard *Z*-scores, so it was possible to compare the results.

Definitions

HPeV- or EV-meningitis was defined as the detection of HPeV or EV in the CSF of a symptomatic patient.

HPeV- or EV-infection elsewhere was defined as the detection of HPeV or EV in viral culture or RT-qPCR of nasopharyngeal, blood, urine or feces specimens of a symptomatic patient. If CSF was collected, than this was negative for HPeV/EV.

No pathogen detected was defined as no detection of HPeV, EV or any other pathogen in viral or bacterial culture or RT-qPCRs of all specimens.

Suspect motor neurodevelopmental delay (below the norm) was defined as a *Z*-score ≤ -1 for the BSID-III or M-ABC-2-NL.

Data collection and statistical methods

Data on demographics, presenting symptoms, presence of extra pediatric physical therapy, growth measurements, and the results of motor tests were captured in an SPSS database. Statistical analyses were performed with SPSS 23.0 (Windows Inc, Chicago, IL, USA). A Chi-squared test or the Fisher's exact test was used for the analysis of categorical data and the student *t*-test for continuous variables with normal distribution. The Mann-Whitney test was used for continuous variables without a normal distribution. The one-way analysis of variance (ANOVA) was used to test the mean of three or more independent groups. *Z*-scores were analyzed as continuous data with the ANOVA and as categorical data (suspect motor neurodevelopmental delay or normal) with the Chi-squared test. Continuous variables are presented as median or mean, with range. A p-value < 0.05 was set as level of significance.

Ethical considerations

The patients and parents/legal guardians received both oral and written information about the study at inclusion and were required to sign a written informed consent form before enrolment. Approximately, 24 months after inclusion the children and their parents were invited for a follow-up visit. The Medical Ethics Committee of each participating center approved the study. The study registration number is NL21361.008.07.

Results

Baseline data

The motor neurodevelopment of 157 children was analyzed. The baseline data of the study children are described in Table 4.2. 114 (73%) children had an HPeV- or EV-infection and in 43 (27%) children no pathogen was detected. The children with an HPeV- and EV-infection were significantly younger, more frequently hospitalized and had a longer duration of hospitalization than those in whom no pathogen was detected.

HPeV- and EV-infected children

Table 4.3 shows the baseline characteristics of the HPeV- versus the EV-infected children. Children with an HPeV-infection did not differ from those with EV-infection, except for a significant lower white bloodcell count (WBC). Of the 114 children with an HPeV- or EV-infection, 56 (49%) had meningitis and 58 (51%) infection elsewhere (gastro-enteritis n = 15, respiratory tract infection n = 3, generalized viral infection n = 40). There were no other CNS involvements except for meningitis. The children with meningitis were significantly younger, more often admitted to the hospital and had a longer duration of hospitalization than those with an infection elsewhere. Of the children with meningitis, 19 had CSF pleocytosis (44%) and 24 (56%) did not. In 13 children there was not enough CSF left, to determine the presence of pleocytosis. Children with meningitis without pleocytosis were significantly younger than those with pleocytosis (2 days vs 456 days, p = 0.049) and those without pleocytosis had more often an EV-meningitis than HPeV-meningitis (17 vs 7 children, p = 0.012, Cramer's V 0.392). There were no differences in gender, hospitalization, duration of hospitalization, blood CRP level or WBC between children with and without pleocytosis.

Motor neurodevelopment

Of the 157 children with complete data records at 24-months follow-up, 16 (10%) had a suspect motor neurodevelopmental delay (Figure 4.1). Of these 16 children 7 (44%) had a meningitis, 6 (38%) an infection elsewhere and in 3 (19%) no pathogen was detected at inclusion (p = 0.67). There was no significant difference in the total motor neurodevelopmental outcome between children with a meningitis and those with an infection elsewhere (p = 0.72) or those with no pathogen detected (p = 0.51). There were also no significant differences in the GMF- and FMF-neurodevelopment of the children.

Table 4.2: Baseline characteristics

	Included patients (*n* = 157)	HPeV/EV positive (*n* = 114)	No pathogen (*n* = 43)	*p*-value
Mean age at inclusion in days (range)	431 (2–4677)	319 (3–4677)	728 (2–4623)	< 0.001*
Median age at inclusion in days (range)	55 (2–4677)	49.5 (3–4677)	247 (2–4623)	< 0.001*
Male/female ratio	101/56 = 1.8	76/38 = 2.0	25/18 = 1.4	0.32
Diagnosis (%)				
HPeV/EV meningitis	56 (36%)	56 (49%)	0 (0%)	< 0.001*
HPeV/EV infection elsewhere	58 (37%)	58 (51%)	0 (0%)	
No pathogen detected	43 (27%)	0 (0%)	43 (100%)	
No. of hospitalized children (%)	130 (83%)	101 (89%)	29 (67%)	0.002*
Median duration of hospitalization in days (range)	3 (0.5–11)	3 (1–6)	4 (0–11)	0.011*
CRP level [mg/l] (range)	16.5 (< 1–181)	17.3 (< 1–181)	13.6 (< 1–120)	0.625
Blood WBC count [*10^e9/l] (range)	10.3 (2.7–27.2)	10.0 (2.9–27.2)	11.1 (2.7–25.6)	0.762
Motor development[a]				
Total patients with suspect total motor neurodevelopmental delay (%) [mean *Z*-score]	16 (0.162)	13 (11%) [0.0192]	3 (7%) [0.393]	0.560 [0.200]
Total patients with suspect GMF-neurodevelopmental delay (%) [mean *Z*-score]	26 (0.0192)	21 (19%) [0.0142][b]	5 (12%) [0.0324][d]	0.298 [0.766]
Total patients with suspect FMF-neurodevelopmental delay (%) [mean *Z*-score]	9 (0.655)	7 (6%) [0.629][c]	2 (5%) [0.699][d]	1.000 [0.765]

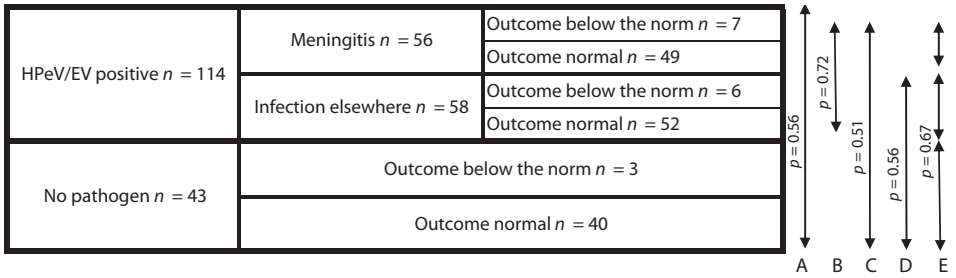
* Significant. [a] *Z*-score ≤ 1 was considered as suspect motor neurodevelopmental delay as a categorical variable. [b] 6 patients missing. [c] 4 patients missing. [d] 2 patients missing.

Table 4.3: Comparison of baseline data of HPeV and EV infected children

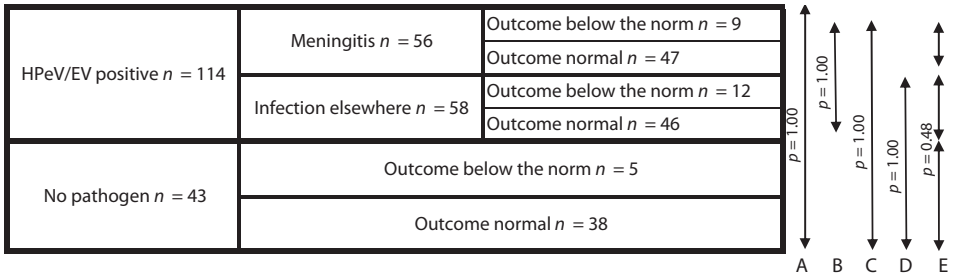
	HPeV ($n = 25$)	EV ($n = 89$)	p- value	HPeV/EV meningitis ($n = 56$)	HPeV/EV infection elsewhere ($n = 58$)	p-value
Mean age at inclusion in days (range)	344 (8–4677)	312 (3–3784)	0.850	247.27 (3–3784)	387.72 (3–4677)	0.001*
Median age at inclusion in days (range)	68 (8–4677)	45 (3–3784)	0.850	35.5 (3–3784)	75 (3–4677)	0.001*
Male/female ratio	16/9 = 1.8	60/29 = 2.1	0.749	37/19 = 1.9	39/19 = 2.1	0.895
Diagnosis						
Meningitis (%)	10 (40%)	46 (52%)	0.302	46 (82%)	42 (72%)	0.216
Infection elsewhere (%)	15 (60%)	43 (48%)		10 (18%)	16 (28%)	0.216
No. of hospitalized children (%)	23 (92%)	78 (88%)	0.730	55 (98%)	46 (79%)	0.002*
Median duration of hospitalization in days (range)	3 (1–5)	3 (1–6)	0.198	3 (2–6)	3 (1–5)	0.032*
CRP level [mg/l] (range)	20.9 (< 1–127)	16.4 (< 1–181)	0.619	9.3 (< 1–62)	26.3 (< 1–181)	0.112
Blood WBC count [*10^{e}9/l] (range)	7.6 (2.9–16.9)	10.6 (4.4–27.2)	0.020*	9.9 (2.9–21.2)	10.2 (3.6–27.2)	0.962
Motor development[a]						
Number patients with suspect total motor neurodevelopmental delay (%) [mean Z-score]	3 (12%) [-0.0300]	10 (11%) [0.0340]	1.000 [0.831]	7 (13%) [0.337]	6 (10%) [-0.253]	0.717 [0.233]
Number patients with suspect GMF-neurodevelopmental delay (%) [mean Z-score]	6 (27%) [-0.243][b]	15 (17%) [0.0801][b]	1.000 [0.243]	9 (18%) [0.201][d]	12 (21%) [-0.153][c]	1.000 [0.171]
Number patients with suspect FMF-neurodevelopmental delay (%) [mean Z-score]	1 (5%) [0.500][b]	6 (7%) [0.6615][c]	0.365 [0.638]	3 (6%) [0.557][e]	4 (7%) [0.694]	0.655 [0.513]

* Significant. [a] Z-score ≤ 1 was considered as suspect motor neurodevelopmental delay as a categorical variable. [b] 3 patients missing. [c] 1 patient missing. [d] 5 patients missing. [e] 4 patients missing.

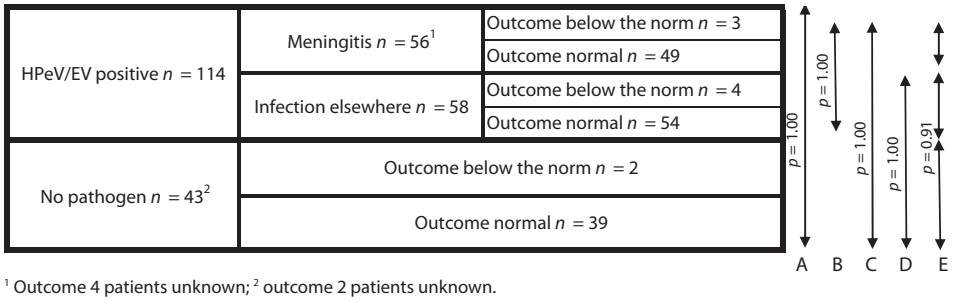
A. Total motor neurodevelopmental outcome



B. GMF-neurodevelopmental outcome



C. FMF-neurodevelopmental outcome



[1] Outcome 4 patients unknown; [2] outcome 2 patients unknown.

Figure 4.1: Motor neurodevelopmental outcome of the 157 included children.
Part A shows data for the total motor neurodevelopmental outcome. Part B shows data for the GMF-neurodevelopmental outcome and part C for the FMF-neurodevelopmental outcome. The motor neurodevelopmental outcome is based on the *Z*-score and a *Z*-score ≤ 1.0 was considered as suspect motor neurodevelopmental delay (below the norm). On the right of each figure are the *p*-values for each comparison. A: HPeV/EV versus no pathogen detected; B: meningitis versus infection elsewhere; C: meningitis versus no pathogen detected; D: infection elsewhere versus no pathogen detected; E: meningitis versus infection elsewhere versus no pathogen detected.

Of the 114 HPeV- or EV-infected children, 89 (78%) were EV-positive and 25 (22%) were HPeV positive. 10 (11%) of the EV-positive children had a suspect motor neurodevelopmental delay (Figure 4.2). Six (60%) of these children had a meningitis. There were no significant differences in the motor neurodevelopment of children with an EV-infection between those

A. Total motor neurodevelopmental outcome

HPeV-positive $n = 25$	Meningitis $n = 10$	Outcome below the norm $n = 1$[1]	$p = 1.00$ (A)
		Outcome normal $n = 9$[2]	
	Infection elsewhere $n = 15$	Outcome below the norm $n = 2$[3]	$p = 0.72$ (B)
		Outcome normal $n = 13$[4]	
EV-positive $n = 89$	Meningitis $n = 46$	Outcome below the norm $n = 6$[5]	$p = 0.74$ (A)
		Outcome normal $n = 40$[6]	
	Infection elsewhere $n = 43$	Outcome below the norm $n = 4$[7]	
		Outcome normal $n = 39$[8]	

CV: coxsackievirus; EV: enterovirus; E: echovirus.
[1] HPeV-3: 1.
[2] HPeV 3: 8, not typeable: 1.
[3] HPeV 1: 1, not typeable: 1.
[4] HPeV-1: 3, HPeV-3: 4, HPeV-4: 1, HPeV-6: 1, not typeable: 4.
[5] E-30: 2, E-17: 1, E-18: 1, E-25: 1, not typeable: 1.
[6] E-6: 4, CV-B3: 3, CV-B5: 3, E-30: 3, E-21: 2, E-25: 2, CV-B2: 2, CV-A9: 1, CV-B4: 1, E-3: 1, E-9: 1, E-11: 1, E-13: 1, E-16: 1, E-19: 1, EV-71: 1, not typeable: 12.
[7] Not typeable: 4.
[8] E-30: 7, CV-A9: 3, CV-B3: 2, E-6: 2, E-11: 2, E-16: 2, E-18: 2, CV-A10: 1, CV-A16: 1, CV-B1: 1, CV-B2: 1, E-7:1, E-9: 1, E-13:1, E-21: 1, not typeable: 11.

B. GMF-neurodevelopmental outcome

HPeV-positive $n = 25$	Meningitis $n = 10$	Outcome below the norm $n = 2$	$p = 1.00$ (A)
		Outcome normal $n = 8$	
	Infection elsewhere $n = 15$	Outcome below the norm $n = 4$	$p = 0.67$ (B)
		Outcome normal $n = 11$	
EV-positive $n = 89$	Meningitis $n = 46$	Outcome below the norm $n = 7$	$p = 0.67$ (A)
		Outcome normal $n = 39$	
	Infection elsewhere $n = 43$	Outcome below the norm $n = 8$	
		Outcome normal $n = 35$	

C. FMF-neurodevelopmental outcome

HPeV-positive $n = 25$	Meningitis $n = 10$[1]	Outcome below the norm $n = 0$	$p = 1.00$ (A)
		Outcome normal $n = 7$	
	Infection elsewhere $n = 15$	Outcome below the norm $n = 1$	$p = 1.00$ (B)
		Outcome normal $n = 14$	
EV-positive $n = 89$	Meningitis $n = 46$[2]	Outcome below the norm $n = 3$	$p = 1.00$ (A)
		Outcome normal $n = 42$	
	Infection elsewhere $n = 43$	Outcome below the norm $n = 3$	
		Outcome normal $n = 40$	

[1] Outcome 3 patients unknown; [2] outcome 1 patient unknown.

Figure 4.2: Motor neurodevelopmental outcome of the 114 HPeV- and EV-positive children.
Part A shows data for the total motor neurodevelopmental outcome. Part B shows data for the GMF neurodevelopmental outcome and part C for the FMF neurodevelopmental outcome. The motor neurodevelopmental outcome is based on the *Z*-score and a *Z*-score ≤ 1.0 was considered as suspect motor neurodevelopmental delay (below the norm). On the right of each figure are the *p*-values for each comparison. A: meningitis versus infection elsewhere; B: HPeV-positive versus EV-positive.

with and without meningitis ($p = 0.74$). Of the 25 children with an HPeV-infection, 3 (12%) had an impaired motor neurodevelopment of which 1 had meningitis and 2 an infection elsewhere ($p = 1.00$). There were also no significant differences in the GMF- and FMF-neurodevelopment between children with an HPeV- and EV-infection.

All the above findings did not change after correcting for age of onset, gender and education level of the parents in a multivariate regression analysis model. There was no clear HPeV- and EV-genotype preference for predicting a suspect motor neurodevelopmental delay. We also examined if the patients had specific early intervention from their parents or a pediatric physical therapist during the follow-up, but we could not find a statistical difference between the different groups of patients.

Discussion

This prospective, multicenter, cohort study, did not find any significant differences in the motor neurodevelopment of children with HPeV- or EV-meningitis compared to those without meningitis and those without any proven infection. There was no difference in the prevalence of motor neurodevelopmental delay, 24 months after infection in children with HPeV- (12%) or EV- (11%) infection, compared to those (12%) in whom no pathogen was detected. These findings are important, as this is the first cohort study with a relatively long-term follow-up evaluation of the motor function neurodevelopment of a large group of non-premature born children following HPeV-infection. In recent years, HPeV has progressively become an important infectious pathogen in pediatric populations worldwide, since it was first described in the early 1990s.[17] Though there have been four previous reports on neurodevelopment of children with HPeV-infection, this is the first study to conduct such a long-term follow-up of their motor neurodevelopment, in a large cohort of Western children of non-Asian origin.[1-4] All previous studies included less than 20 children. Verboon-Maciolek et al. described 10 neonates who presented at 25-41 weeks post-menstrual age with seizures caused by an HPeV-infection.[3] The neurodevelopment was impaired in 4 (40%) children (cerebral palsy in 1, a suspect outcome at 18 months in 1, learning disabilities at 7 years of age in 1 and epilepsy in 1). One infant was only followed-up for 9 months. In another study, Verboon-Maciolek et al. described 11 neonates with HPeV-infection, of which 3 (27%) infants had a neurodevelopmental delay.[4] A possible explanation for the higher rate of impaired neurocognitive development in their study could be that the children were partly preterm, were infected at a younger age and probably more

severely ill (NICU hospitalization) compared to the children in our study. Preterm children were excluded in the present study. In addition, the children in this present study were presumably less severely ill (no ICU admissions). This makes our study population a better representation of the population of children seen in the majority of hospitals in the country then the previous ones. Britton et al. recently described 13 pediatric cases with suspected HPeV-encephalitis, followed up for a period of 12 months. All these children were infected at an age < 2 months, with a median age of 13 days. They used only questionnaires and telephone interviews of parents to assess the motor neurodevelopment.[1] Nine children had HPeV-encephalitis and 4 an HPeV-infection without encephalitis. Five (63%) of 8 infants with HPeV-encephalitis showed neurodevelopmental dysfunction: 3 severe (2 cerebral palsy, 1 central visual impairment) and 2 showed concern in gross motor neurodevelopment. One child was lost to follow-up. Seven of the children with HPeV-encephalitis were girls, 5 preterm, 8 admitted to an ICU and 8 had seizures, indicating that they differed from our study population. Moreover, they were not tested by a pediatric physical therapist or with more objective tests for motor neurodevelopment.

Vergnano et al., retrospectively identified 50 children with an HPeV-infection of which in 19 infants follow-up data were available.[2] Developmental delay was evident in 6 (32%) of the 19 infants. Three infants had identifiable neurological sequelae. One developed cerebral palsy and visual impairment, one had speech delay and a third generalized hypotonia. All three presented with seizures and were admitted to the PICU. Neither the test method used to assess their motor neurodevelopment nor the health worker, who conducted the test, were clearly described.

Several authors have reported a delayed neurodevelopment after EV-71 CNS-infection.[5-9] EV-71-infection is more endemic in Asia, especially in China and Taiwan. They are less prevalent in Europe and the US. Only one patient in our study had an EV-71-infection. Though this patient had an EV-meningitis, he did not develop a fine motor function neurodevelopmental delay at 24 months follow-up (He refused to perform the gross motor activities at this follow-up moment).

The present study is the largest to describe the motor neurodevelopmental outcomes of children after EV-infection, not caused by EV-71. There are a few other published data on the outcome of motor neurodevelopment in children after EV-meningitis, not caused by EV-71. However, these are relatively old studies with small numbers of pediatric patients. In 1975, Sells et al. performed a controlled follow-up study in 19 children aged 2.5–8 years, hospitalized with documented EV-CNS-infection (aseptic meningitis in 9, meningoencephalitis in 9 and

acute cerebellar ataxia in 1) 17–67 months before evaluation.[18] Three children (16%) had definite neurologic impairment, five (26%) had possible impairment, and 11 (58%) had no impairment. In that study, they performed the motor function testing with an older version of the BSID and the motor function domain of the test was conducted by a psychologist. Wilfert et al. performed a case-control study of 9 children with an EV-meningitis during the first three months of life in 1981.[19] Receptive vocabulary testing suggested that the receptive language functioning of the group with meningitis was less than the control group. Verboon-Maciolek et al. described 21 neonates with an EV-infection of which one had a neurodevelopmental delay.[4] Unfortunately, they did not describe which tests they used for the motor neurodevelopment testing.

In summary, we report a lower percentage of suspect motor neurodevelopmental delay than previous studies in children with HPeV- or EV-infection, including meningitis. Among the factors which may partly explain these differences in finding are differences in genetic predisposition, the heterogeneity in the study sizes, differences in gender and race, virus genotypes, severity of disease, time from symptoms to presentation at the hospital, duration of follow-up and type of instruments used for motor neurodevelopment testing. We cannot exclude that the higher rate of hospitalization of the group of children with meningitis is caused by the tendency of some pediatricians to hospitalize younger, ill children because this group of children was younger than those without meningitis. Compared to most previous studies on this subject, the children included in our study are more representative for the general Dutch population and the pediatric physical therapist who conducted the study was blinded to the clinical diagnosis of the study children. This further reduced the risks of a detection bias in the study population.

One of the limitations of this study may be that only 10 children had HPeV-meningitis, making it more difficult to conduct group analyses in those with HPeV-infection. However, this problem seems to have a limited effect on the results since we neither saw a trend to significance in any of the analyses performed. Another limitation is that only the results of motor function test 24 months after infection are reported. Therefore it is not possible to detect temporary motor neurodevelopmental delay that may have occurred shortly after infection. However, the major study objective was to describe the medium term rather than the short term effect on motor function in children.

In conclusion, we could not find a statistical significant difference in the motor neurodevelopment of children 24 months after an HPeV- or EV-infection.

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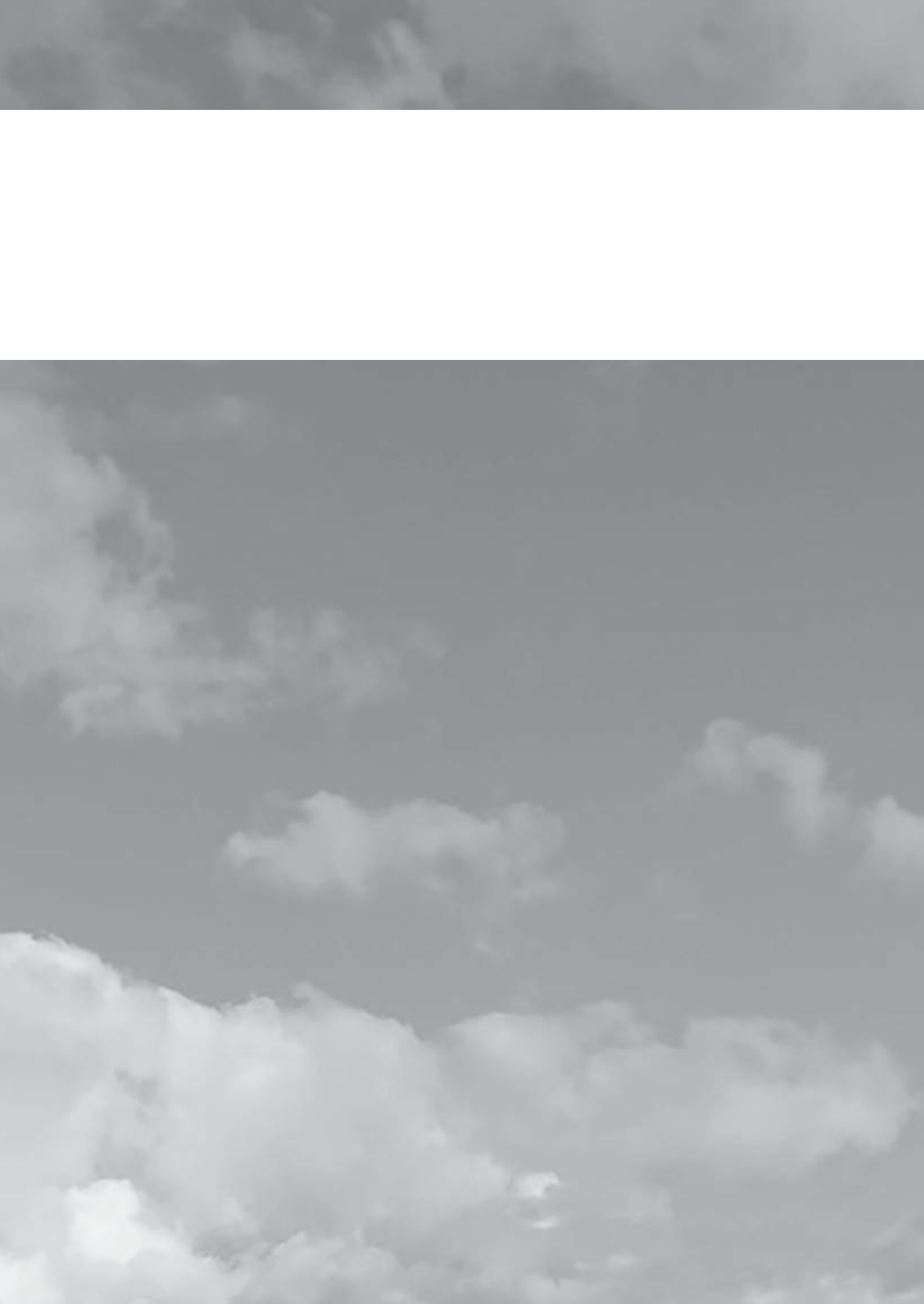

Part 4

Longitudinal assessment

The impact of Parechovirus CNS infection on gross and fine motor function neurodevelopment

5

Longitudinal association between human Parechovirus central nervous system infection and gross motor neurodevelopment in young children

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Abstract

Background: A paucity of studies investigated the association between human parechovirus (HPeV) central nervous system (CNS) infection and motor and neurocognitive development of children. This study describes the gross motor function (GMF) in young children during 24 months after HPeV-CNS-infection compared with children in whom no pathogen was detected.

Methods: GMF of children was assessed with Alberta infant motor scale, Bayley scales of infant and toddler development or Movement assessment battery for children. We conducted multivariate analyses and adjusted for age at onset, maternal education and time from infection.

Results: Of 91 included children, onset < 24 months of age, 11 had HPeV-CNS-infection and in 47 no pathogen was detected. Nineteen children were excluded due to the presence of other infection, preterm birth or genetic disorder and in 14 children parents refused to consent for participation. We found no statistically significant longitudinal association between HPeV-CNS-infection and GMF (β = -0.53; 95% CI = -1.18 to 0.07; $p = 0.11$). At 6 months, children with HPeV-CNS-infection had suspect GMF-neurodevelopmental delay compared with the non-pathogen group (mean difference = 1.12; 95% CI = -1.96 to -0.30; $p = 0.03$). This difference disappeared during 24-months follow-up and, after adjustment for age at onset, both groups scored within the normal range for age. Maternal education and time from infection did not have any meaningful influence.

Conclusion: We found no statistically significant longitudinal association between HPeV-CNS-infection and GMF-neurodevelopment during the first 24-months follow-up. Children with HPeV-CNS-infection showed a suspect GMF-neurodevelopmental delay at 6-months follow-up. This normalized during 24-month follow-up.

Introduction

Human parechovirus (HPeV) are becoming a major viral cause of central nervous system infection in young children.[1-4] The clinical manifestation of HPeV-CNS-infection depends on the location of infection, genotype and age at onset.[1-5] HPeV genotype 3 (HPeV-3) has been associated with severe CNS-infections in young children and neonates are at highest risk of severe HPeV-CNS-infection.[1-5] The HPeV-3 receptor may facilitate entry of HPeV-3 to neonatal central nervous system cells[6] and may be responsible for white matter damage, resulting in functional disorders such as cerebral palsy, neurodevelopmental disorder, gross motor function (GMF) delay and retardation.[7-9]

Early detection of GMF-neurodevelopmental delay is important because it predicts generalized neurodevelopmental delay in young infants[10] and allows the initiation of intervention programs to prevent permanent motor functional neurodevelopmental delay.[11] Studies[7-9,12,13] that investigated the effect of HPeV-CNS-infection on GMF in the first 24 months after infection have generally been inconclusive. While some studies found HPeV-CNS-infection to cause temporary neurodevelopmental disorders,[8,12,13] others reported persistent abnormalities.[7-9] These studies have been based on cross-sectional analyses, that lacked the longitudinal follow-up necessary to detect intra-individual variations of GMF with time. In addition, most of the results have been based on the use of less discriminative tests for GMF such as the Griffith neurodevelopmental scale,[7] parental self-report,[8] or routine neurologic examination.[7,8,9,12] These measurements may lack responsiveness to evaluate changes in GMF with time. The use of standardized and validated GMF-assessments is more sensitive in detecting minor GMF-neurodevelopmental delay and allow an evaluation of GMF-neurodevelopment with time.

The objective of this study was to determine the longitudinal association between HPeV-CNS-infection and GMF-neurodevelopment during a period of 24 months after infection. We hypothesized that children with an HPeV-CNS-infection would show significant more suspect GMF-neurodevelopmental delay during the first 24 months after infection, compared with those without infection.

Methods

Participants

This prospective cohort study was part of a multicenter study that investigated the clinical presentation, diagnostic methods and prognosis of HPeV-CNS-infections in a cohort of

Caucasian Dutch children (Netherlands trial registry #NTR3193). Details of the study design have previously been reported.[14] Between March 2008 and September 2011, we consecutively recruited febrile children aged < 24 months from the pediatric emergency or outpatient departments of three general hospitals in the Netherlands, Elisabeth hospital Tilburg, Tweesteden hospital Tilburg, Amphia hospital Breda. The in- and exclusion criteria are described in Table 5.1. The parents or legal guardians of the eligible children received oral and written information about the study and were invited to participate. Only the children whose parents/legal guardians signed a written informed consent were included in the study. In all included children we collected nasopharyngeal swab, blood, urine and feces specimens for viral or bacterial culture and viral reverse-transcriptase real time quantitative polymerase chain reaction (RT-qPCR).[14,15] Children with clinical signs of CNS-infection underwent a lumbar puncture to collect cerebrospinal fluid (CSF) specimen for viral and bacterial culture and RT-qPCR for neurotropic viral pathogens including HPeV, enterovirus (EV), human herpes virus, and varicella zoster virus. The medical ethics committee of each participating center approved the study (NL-21361.008.07).

Table 5.1: Inclusion and exclusion criteria of the study children

Inclusion criteria
Children 0–24 months of age with at least one of the following clinical signs and symptoms:
1. Fever (temperature ≥ 38.0°C or ≥ 100.4°F)
or
2. Clinical signs or symptoms of meningitis including headache, photophobia, nuchal rigidity, irritability, lethargy, nausea, vomiting, drowsiness, positive sign of Kernig or Brudzinsky[a]
or
3. Other clinical signs and symptoms of infection: hypothermia, vomiting, diarrhea, anorexia, cough, myalgia, rash, hypovolemia or shock[b]
Exclusion criteria
1. Other proven infectious cause of the clinical symptoms
2. Other non-infectious cause of clinical symptoms: e.g., neoplasm, auto-immune diseases, rheumatic diseases, endocrinology diseases, gastro oesophageal reflux
3. Known severe psychomotor retardation, metabolic diseases with neuromuscular and/or cognitive abnormalities
4. Intra-uterine and perinatal problems or traumatic head injury
5. Preterm birth (gestational age < 37 weeks)
6. Children ≥ 24 months of age

[a] At least 2 of these signs or symptoms must be present. [b] At least 3 of these signs or symptoms must be present.

Laboratory methodology

An aliquot (200 µL) of nasopharynx, blood, urine, feces or CSF specimen was used to extract (viral) RNA as described previously.[14] The isolated (viral) RNA was analyzed for the presence of HPeV using an HPeV-specific RT-qPCR.[16] Virus was cultured on confluent layers of tertiary cynomolgus monkey kidney cells; 0.25 mL of clinical specimen was inoculated in the cells for 1 hour, after which 1 mL of culture medium was added and maintained at 37°C on roller drums. These were examined daily for 14 days for a cytopathic effect, before genotyping of the virus isolates was carried out by standard procedures.[14]

Study groups

Based on the results of the viral diagnostics of their body specimens, study children were subdivided in two groups: those in whom HPeV was detected in CSF specimen (HPeV-CNS-infection group) and those in whom no HPeV or other pathogen was detected in any of their body specimens (non-pathogen group). If both HPeV and other pathogen were detected from CSF specimen of the same child, this child was excluded from the study.

Procedure follow-up

All included children were invited for GMF-testing at the pediatric outpatient departments at 6, 12 and 24 months after inclusion. To prevent differential measurement bias, parents/legal guardians were instructed not to reveal their child's diagnosis to the pediatric physical therapist assessing the GMF-neurodevelopment. Before starting the GMF-assessment, the pediatric physical therapist classified alertness and cooperation of the child as either alert, cooperative, active movement, sleepy, inactivity, struggling or crying. If a child did not cooperate during the test, the GMF-assessment was postponed and rescheduled to a new date. The body mass, length and head circumference of each child was recorded. Parents/legal guardians completed a questionnaire on their child's general health, recent traumatic head injury, medication use, and on any early intervention (e.g., pre-speech and GMF-training). According to Statistics Netherlands (Centraal Bureau voor de Statistiek, CBS)), maternal education was classified as low (primary school or lower vocational education), middle (middle vocational education) and high (higher vocational education or university degree).[17]

Gross motor function neurodevelopment

The GMF-neurodevelopment was assessed with three norm-referenced observational and performance-based instruments: the Alberta infant motor scale (AIMS);[18] the Bayley scales of infant and toddler development version-3 (Bayley-3-NL);[19-21] and the Movement assessment battery for children version-2 (M-ABC-2 NL).[22]

The AIMS[18] is suitable for detecting minor GMF-neurodevelopmental delay in children aged between 4 and15 months.[23-25] The Bayley-3-NL test has been validated in Dutch children aged from 16 days to 42.5 months.[20,21] We utilized only it's gross motor domain. The GMF of children aged > 42.5 months was assessed with the domain static and dynamic balance of the M-ABC-2-NL.[22] All these instruments are suitable for Dutch children and can detect minor motor neurodevelopmental delay in children.[18-23]

All GMF raw scores were converted to age adjusted standard deviation (*SD*) scores (*Z*-scores), according to the technical manual of each instrument. Based on age specific population norms a suspect GMF-neurodevelopmental delay was defined as *Z*-score of ≤ -1 (equivalent to ≤ -1 *SD*).[18-20,22]

Statistical analyses

Statistical analyses were performed using R, version 3.4.0 (R Foundation for statistical computing platform, Vienna, Austria). Descriptive analyses were used to compare baseline characteristics between children with HPeV-CNS-infection and children in the non-pathogen group. Fisher exact test was used for the analyses of categorical variables and the Independent *t*-test for continuous variables or the Mann-Whitney *U* test in case of non-normally distributed continuous variables. We used the R-package linear and nonlinear mixed effects models (NMLE)[26] to determine the longitudinal association between HPeV-CNS-infection and GMF-neurodevelopment. Cross tabulations by time for each child were utilized to investigate the differences in intercepts and slopes. We checked assumptions of normality and constant variance of residuals and used 1-way analysis of variance to compare the model fit. The linear mixed effects model uses the maximum likelihood method. This implies that missing data are included as information, therefore, we did not impute missing data.

Based on previous research[1-5,11] and modelling causal relationships using directed acyclic graphs, we expected that a child's age at onset[1-5] and maternal education[11] would distort the longitudinal association between HPeV-CNS-infection and GMF-neurodevelopment. Therefore, we included these variables as covariates in the model. Because of the limited sample size, we entered these covariates in 2 separate models We developed 1 unadjusted

model and 2 adjusted models respectively for age at onset and maternal education. Maternal education was dichotomized as either low and middle versus high. In addition, we adjusted for time from infection in each of the models. A *p*-value below 0.05 was considered statistically significant for all comparisons.

Results

In total 91 children were eligible for participation. Nineteen children were excluded due to other viral (rotavirus, norovirus or adenovirus in 6-, rhinovirus in 3-, and coronavirus in 1 child) or bacterial (bacterial urinary tract infection in 1 child) infection, 7 due to preterm birth and 1 due to a genetic disorder. The parents/legal guardians of 14 children refused to consent for their participation.

Table 5.2 represents the baseline characteristics of the included children (n = 58). Eleven children had a HPeV-CNS-infection. In 47 children no pathogen was detected. Of the children with HPeV-CNS-infection, HPeV-3 genotype was detected in 9 children (82%) and HPeV-1 in 1 child (9%). In 1 child (9%) there was an indeterminate genotype.

Table 5.2: Characteristics at baseline of the children with human parechovirus central nervous system infection and children in whom no pathogen was detected

Characteristics	HPeV-CNS-infection *n* = 11	Non-pathogen *n* = 47
Gender		
Boys (%)	10 (91)	28 (60)
Type HpeV		
HPeV-3 (%)	9 (82)	
HPeV-1 (%)	1 (9.0)	
Indeterminate genotype of HPeV (%)	1 (9.0)	
Age at onset in days		
Mean (*SD*)	39 (29)*	187 (187)*
Min/max	13/114	2/730
Hospitalization in days		
Mean (*SD*)	3.6 (0.8)	3.9 (2.3)
Min/max	3.0/5.0	0.0/11.0
Maternal education		
Low (%)	1 (9.1)	4 (8.5)
Middle (%)	6 (54.5)	12 (25.5)
High (%)	4 (36.4)	29 (61.7)
Missing (%)	0 (0.0)	2 (4.3)

* $p < 0.05$: statistically significant difference between groups; Fisher exact test; Independent *t*-test or Mann-Whitney *U* test. *N*: number of included children.

The groups did not differ in baseline characteristics, except for the age at onset and gender. Children with an HPeV-CNS-infection were younger [mean age (*SD*) = 39 (29)] than those in the non-pathogen group [mean (*SD*) = 187 (187); (p = 0.01]. There were more boys with HPeV-CNS-infection (91%) than in the non-pathogen group (60%); (p = 0.08).

The groups did not differ in anthropometrical parameters, general health condition, presence of recent head injuries and use of medication during the follow-up visits. Two children in the HPeV-CNS-infection group, and 5 children in the non-pathogen group received early intervention for positional nonsynostotic plagiocephaly. One child in the non-pathogen group had neonatal transient abnormal intra-uterine foot position.

Results of GMF-assessments

Table 5.3 summarizes the results of the *Z*-scores for gross motor function neurodevelopment at 6-, 12- and 24-months follow-up. Six months after infection, children with an HPeV-CNS-infection had a significant lower mean GMF *Z*-score [mean *Z*-score (*SD*) = -1.69 (1.05)], suggesting a suspect GMF-neurodevelopmental delay for age. In contrast, children in the non-pathogen group did not have clinically relevant suspect GMF-neurodevelopmental delay for age (mean *Z*-score (*SD*) = -0.58 (1.20)). At 12 and 24 months after inclusion children in both groups showed mean GMF *Z*-scores within the normal range for age.

The mean difference in GMF *Z*-scores between the two groups was clinically relevant (mean difference *Z*-score = 1.12; 95% Confidence Interval (CI) = -1.96 to -0.30; p = 0.03) at 6 months, but not at 12 and 24 months (respectively mean difference *Z*-score = 0.33; 95% CI = -0.10 to 1.65; p = 0.60 and 0.44; 95% CI = -1.21 to 0.34; p = 0.18).

Records of at least 2 follow-up visits were completed in 33 children (57%), including 9 in the HPeV-CNS-infection group (82%). Of these 27.3% attended all follow-up visits, 54.5% attended 2 follow-up visits and 18.2% attended 1 follow-up visit. In the non-pathogen group 6.4% attended all follow-up visits, 44.7% attended 2 follow-up visits and 48.9% attended 1 follow-up visit. All missing data were missing completely at random. Reasons for lost to follow-up were relocation of the family outside our catchment area, divorce of parents with one parent moving away with the child to unknown address or parents/legal guardians were too busy to attend follow-up appointments.

Table 5.4 presents the longitudinal association between HPeV-CNS-infection and GMF-neurodevelopment. The longitudinal analysis showed no statistically significant longitudinal

association between HPeV-CNS-infection and GMF-neurodevelopment. Compared with the non-pathogen group, children in the HPeV-CNS-infection group had less average mean GMF *Z*-score of 0.53 (β = -0.53; 95% CI = -1.18 to 0.07; $p = 0.11$). Adjustment for the age at onset weakend the association between HPeV-CNS-infection and GMF-neurodevelopment

Table 5.3: Comparison of gross motor function neurodevelopment of children with human parechovirus central nervous system infection and children in whom no pathogen was detected

Follow-up	HPeV-CNS-infection	Non-pathogen	Mean difference (95% CI)	*p*-value
6 months				
n (%)	9 (81.8)	24 (51.1)		
Mean *Z*-score (*SD*)	-1.69 (1.05)	-0.58 (1.20)	1.12 (-1.96 to -0.30)	0.03*
12 months				
n (%)	5 (45.5)	18 (38.3)		
Mean *Z*-score (*SD*)	0.19 (0.83)	-0.14 (1.34)	0.33 (-0.10 to1.65)	0.60
24 months				
n (%)	9 (81.8)	32 (68.1)		
Mean *Z*-score (*SD*)	-0.22 (1.19)	0.21 (1.00)	0.44 (-1.21 to 0.34)	0.18

$p < 0.05$: statistically significant difference between groups; Independent *t*-test. *n*: number of children attending follow-up visit. Based on age specific population norms a suspect gross motor function neurodevelopmental delay was defined as *Z*-score of ≤ -1 (equivalent to ≤ -1 *SD*).

Table 5.4: Longitudinal association between human parechovirus central nervous system infection and gross motor function neurodevelopment

Determinant	Average slope of *Z*-score (95% CI)	*p*-value
Unadjusted model		
Intercept	-0.24 (-0.59 to 0.12)	0.20
Time from inclusion	0.07 (-0.23 to 0.08)	0.36
HPeV-CNS-infection[a]	-0.53 (-1.18 to 0.07)	0.11
Model adjusted for age at onset		
Intercept	-0.56 (-1.02 to -0.09)	0.02
Time from inclusion	0.08 (-0.23 to 0.08)	0.33
HPeV-CNS-infection[a]	-0.28 (-0.95 to 0.40)	0.42
Age at onset in days	0.01 (-0.01 to 0.01)	0.05
Model adjusted for maternal education		
Intercept	-0.30 (-0.80 to 0.22)	0.26
Time from inclusion	0.07 (-0.08 to 0.23)	0.37
HPeV-CNS-infection[a]	-0.51 (-1.18 to 0.17)	0.15
Maternal education high	0.10 (-0.48 to 0.68)	0.74

$p < 0.05$: statistically significant difference between groups in unstandardized regression coefficient beta (β). The size of association, expressed in the unstandardized regression coefficient beta, showed the average slope of the *Z*-score during follow-up, 6–24 months. [a] Average lower *Z*-score of the HPeV-CNS-infection group during follow-up compared with the non-pathogen group.

(β = -0.28; 95% CI = -0.95 to 0.40; $p = 0.42$). Adjustment for maternal education did not influence the association of HPeV-CNS-infection and GMF-neurodevelopment (β = -0.51; 95% CI = -1.18 to 0.17; $p = 0.15$). Adding the interaction term for time from infection did not influence the longitudinal association between HPeV-CNS-infection and GMF-neurodevelopment.

Discussion

In this study we investigated the GMF-neurodevelopment in young Dutch children with HPeV-CNS-infection and compared this with children in whom no pathogen was detected. To our knowledge this prospective cohort study is the first study that investigated the longitudinal association between HPeV-CNS-infection and GMF-neurodevelopment in Caucasian children with a follow-up period of 24 months after infection and compare this with a non-pathogen group. We adjusted for age at onset, maternal education and time from infection.

The longitudinal analysis showed no statistically significant association between HPeV-CNS-infection and GMF-neurodevelopment in the first 24 months after infection. The group of children with HPeV-CNS-infection had a reduced mean GMF *Z*-score. At 6 months follow-up, children with HPeV-CNS-infection showed a clinically relevant suspect GMF-neurodevelopmental delay compared with the non-pathogen group. However, the clinical significance of this lower score that suggest a temporary GMF-neurodevelopmental delay is unclear, as this difference disappeared within 24 months of follow-up. Adjustment for age at onset weakend the association between HPeV-CNS-infection and GMF-neurodevelopment. These findings are interesting, as suspect GMF-neurodevelopmental delay is one of the most obvious signs of a generalized neurodevelopmental delay in childhood.[10]

Our results are in line with two previous studies supporting the hypothesis of a possible causal relationship between HPeV-CNS-infection and poor short term neurodevelopmental outcome in young children. In a retrospective follow-up study of 10 Norwegian term birth children, 1 child developed upper limb hypotonia in the first weeks after the infection which disappeared within a year.[12] Recently, De Jong et al. described a follow-up of 4 term birth children with HPeV-CNS-infection, of which 1 child had a GMF-neurodevelopmental delay at 6 months follow-up.[13]

In contrast, others found permanent neurological sequelae and neurodevelopmental disorders during a 6 month, or more, follow-up after HPeV-CNS-infection.[7-9] This may be explained by a difference in gestational age of study subjects. The studies by Verboon et al.

(n = 10),[7] Britton et al. (n = 13)[8] and Vergnano et al. (n = 19)[9] included a mixture of pre- and full term birth children (gestational ages 25 to 41 weeks). We excluded preterm birth children, because they are more likely to show GMF-neurodevelopmental delay during the first 18 months of life,[27] and their GMF-neurodevelopment is more unstable over the course of the first 24 months of life.[28]

Differences in severity of disease and the age at onset of the HPeV-CNS-infection may influence GMF-neurodevelopmental delay.[1-5] About 90% of the children in the afore mentioned prospective studies[7,8] were more severely ill at presentation (sepsis-like illness, seizures and encephalitis), were hospitalized in a pediatric intensive care, and had a longer duration of hospitalization than the children who participated in our study. Furthermore, their participants were younger at onset of the infection (range 6–90 days, not corrected for gestational age)[7,8] than the children in our study (range 13–114 days).

We adjusted for age at onset, which weakend the association between HPeV-CNS-infection and GMF-neurodevelopment. Maternal education and environmental factors are known to influence GMF-neurodevelopment.[11] Adjustment for maternal education and time from infection did not influence the association between HPeV-CNS-infection and GMF. We did not adjust for early intervention, none of the children had any, except those with positional nonsynostotic plagiocephaly. Some previous studies on the relationship between HPeV-CNS-infection and GMF-neurodevelopment in children did not compare findings with a group without infection or blinded the assessor of the GMF-test.[7-9,12] We opted for a blinded observation to reduce the likelihood of differential measurement bias. In this multicenter study, we included children from three general hospitals, which makes our findings more applicable to children elsewhere in similar settings.

A strength of this study is the longitudinal assessment of GMF-neurodevelopmental measurements. We conducted linear mixed effects model analyses that incorporated estimates of intra-individual relations across repeated GMF-measurements, a sensitive method for assessing alterations,[29] such as intra-individual differences due to the effect of neural plasticity of a child's brain. The development of the human brain is a dynamic process and the nervous system keeps evolving, even after the first year of life.[30,31] Suspect GMF-neurodevelopmental delay can become reversible with time.[11,30] We opted for a period of 6 months before conducting the first GMF-tests because we assumed that hospitalization may have temporary psychological sequelae in children that might extend over a longer period than immediately after discharge.[32] We chose a follow-up period of 24 months, the time most children achieve independent walking.[10] HPeV-CNS-infection has been shown to

cause white matter injury in neonates[7-9] and is therefore possibly associated with late onset disorders of the brain.[33] Developmental domains, such as language, cognitive and behavioral functions can be more reliably assessed after the age of 24 months.[10] Though suspect GMF-neurodevelopmental delay may be an early sign of generalized neurodevelopmental delay in young infants, it is possible that children with apparently normal outcome at 24 months of age may develop other problems at school-age.[10] Study designs with a longer follow-up period are needed to test these possible late onset developmental disorders after HPeV-CNS-infection.

We strictly defined our inclusion and exclusion criteria, and the children were recruited consecutively to reduce selection bias. In this cohort, there were no children with an HPeV-CNS-infection and other infection, that could have biased our findings. However, the exclusion of children with other infection may reduce the generalizability of our findings. All study children were enrolled from the same source population presenting to the emergency departments of the participating hospitals in the same period.

We used standardized norm-referenced measurements (the AIMS,[18] the Bayley-3-NL[20] and the M-ABC-2-NL[22]) that are sensitive and reliable in detecting minor GMF-neurodevelopmental delay. The GMF-neurodevelopment in the non-pathogen group was within the -1 *SD* (*Z*-score) from the age-specific population norm[18,20,22] while the GMF-neurodevelopment of the HPeV-CNS-infection group was significantly lower than the age-specific population norm at 6 months follow-up, suggesting influence of the infection.

A limitation of our study is that not all participants were assessed at all the time-points during follow-up. This potentially caused less reliable linear mixed effects models. To address this, we used the maximum likelihood method to correct for missing data and assessments that were not equally spaced in time. Most of the children that were lost to follow-up either moved without forwarding contact details, or forgot to appear at the follow-up visits after repeated invitations.

In our study the number of children with HPeV-CNS-infection was low, which may have influenced the GMF-neurodevelopmental results. However, a low number is not unique to this study. Other studies in Western countries also found low numbers of HPeV-CNS infected children.[7-9,12,13]

This study shows no longitudinal association between HPeV-CNS-infection and GMF-neurodevelopment during the first 24 months follow-up. For future studies we recommend to have a longer period of follow-up with considerably more subjects, assessing developmental domains other than GMF, possible in a multicenter study within Europe.

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7

General discussion

Introduction

Parechovirus (PeV) belongs to the family Picornaviridae.[1] PeV's are neurotropic viruses which cause infections of the central nervous system (CNS). Therefore, it is plausible that several serotypes and strains may lead to neurological sequelae and neurodevelopmental delay. Since 2008, the confirmation of PeV-infection using reverse-transcriptase real time quantitative polymerase chain reaction (RT-qPCR) has become more accurate. This made it possible to differentiate between PeV- and EV-infection in daily practice. In 2008, there was a scarcity of literature on the neurological and neurodevelopmental outcome of neonates and young children with PeV-CNS-infection.[2,3] Therefore, we initiated a longitudinal cohort study of neonates and young children with PeV-CNS-infection in 2008 to address these issues.

The results are presented in this thesis. The main objective of this longitudinal follow-up study was to determine the association between PeV-CNS-infection and gross motor function (GMF) neurodevelopmental outcome in neonates and young children during a period of 5 years after the PeV-CNS-infection. We hypothesized that GMF-neurodevelopmental delay is a potential early sign of CNS-infection-associated generalized neurodevelopmental delay and that these children would show more significant suspect GMF-neurodevelopmental delay during the first 5 years after infection. It is known that early detection of neurodevelopmental delay is important for initiating early intervention programmes to optimise the developmental potential.[4,5]

We compared the children with PeV-CNS-infection with peers with enteroviruses (EV)-CNS-infection, PeV-infection-elsewhere in the body (not CNS-infection), EV-infection-elsewhere in the body (not CNS-infection), and peers in whom no pathogen was detected. In addition, we compared the GMF-neurodevelopmental outcomes to the population standard norm. Next to the GMF-neurodevelopmental outcome, we reported on the fine motor function (FMF) neurodevelopmental outcome, a secondary neurodevelopmental outcome we assessed in participating children between 24 months and 5 years after the infection.

We additionally conducted a systematic review with meta-analyses that gives an overview of the results of follow-up studies of children with PeV-CNS-infection, as well as the strengths and limitations of the included studies.[6]

In this general discussion chapter, we will summarize the main findings and conclusions of this thesis and reflect on these. Then, we will report on the strengths and limitations of our studies. Finally, we will provide considerations for the clinical practice and make recommendations for further research.

Main findings

Our most important finding is that throughout the 5-year follow-up period, children with PeV-CNS-infection younger than 3 months showed more suspect GMF-neurodevelopmental delay than the population norm-referenced GMF-score. They also had more suspect GMF-neurodevelopmental delay than peers with EV-CNS-infection, with PeV-infection-elsewhere, EV-infection-elsewhere, and in whom no pathogen was detected. The average GMF-neurodevelopmental performance of the group of children with PeV-CNS-infection improved between 6 and 24 months. At 5 years of follow-up, the GMF-neurodevelopmental performance decreased.

The FMF-neurodevelopment of the group of children with PeV-CNS-infection was lower at 24 months than children with EV-CNS-infection. This normalised with the population standard norm range at 5 year of follow-up.

The poorer average GMF-neurodevelopmental performance at 5 years may be a prelude of generalized neurodevelopmental disorders later in time.

Comparison of our data with other published studies

The studies by Verboon et al. (2008) are the first follow-up studies of a group of neonates with PeV-CNS-infection.[2,3] As from 2011 more follow-up studies were published.[6,7] Many of these included very vulnerable preterm-born infants who required admission to an ICU, or had co-infections or co-morbidity.[2,3,8-15] In contrast to many of these follow-up studies, the neonates and young children in our cohort study were apparently less severely ill. They were recruited from three general hospitals and did not need ICU-care. All included children were full-term born. Those with co-infections or comorbidities that could impact on neurological or neurodevelopmental outcome were excluded. Those included had an uneventful medical history. Unlike most of those studies, we did not find severe neurological and neurodevelopmental abnormalities such as cerebral palsy, motor or mental retardation. We only found suspect GMF-neurodevelopmental delay. Although the GMF-neurodevelopmental delay reached a clinical threshold in only a minority of children, these findings suggest that PeV-CNS-infection may have more severe impact on the GMF-neurodevelopment of neonates and young children than EV-CNS-infection.

Age and PeV-A3-CNS-infection

All children with PeV-CNS-infection in our cohort were younger than 3 months (age-range 7–68 days) at inclusion. All infections but one, were caused by PeV-A3 (in this one child the PeV was not typable). This is in line with existing literature and with the findings of our systematic review on the outcomes of neonates and young children with PeV-CNS-infection, in which the PeV-A3 is the most common PeV genotype infecting CNS.[6,16-19]

In addition to the inclusion of very vulnerable children (infants who were born preterm,[20,21] admission to an ICU, co-infections and/or co-morbidities), differences in geographical location or virulence of PeV-strains may play a role in severity profiles and outcomes.[22] The results of our meta-analyses suggest that the prognosis of the children in Australian studies, which represented a substantial percentage of the total number of children with follow-up outcomes, is worse than in other regions.[8,9,11,13] A substantial number of these Australian children required admission to an ICU.[8,9,11,13] The reason for these differences may be diversity in virulence between Australian and European PeV-strains. We contemplated to analyse the children with PeV-CNS-infection by geographical distribution in order to extract eventual differences in this respect. Unfortunately, this was not possible due to high clinical, methodological and statistical heterogeneity of the included studies. We recommend that future studies take into account the influence of preterm-birth, ICU-hospitalisation, co-infections and co-morbidities on the neurological and neurodevelopmental outcomes.

GMF-neurodevelopmental delay is an early sign for neurodevelopmental disorder later in life

During the follow-up of the children with PeV-CNS-infection aged ≤ 3 months, we observed more suspect GMF-neurodevelopmental delay at 6 months than at 12 and 24 months after infection. This may partly be explained by the fact that in the first year of life, the GMF-neurodevelopment assessment test focuses mainly on postural control.[23,24] At the age of 6 and 12 months the GMF-neurodevelopment is based on single tasks. At 24 months and particularly at 5 years of age, the tasks will gradually be expanded with more balance, visuomotor coordination, rhythm, motor sequencing and power and strength. With age these tasks become more complicated, involving multiple neurodevelopmental domains working together. More cognitive strategies are addressed by multiple tasks. This is even more pronounced in the FMF-neurodevelopment, which has more overlap with cognition.[25] Milder disorders in other neurodevelopmental domains such as cognition, behaviour,

emotional/personal-social and speech and language, may manifest several years later.[26,27] This is supported by the outcome of the meta-analyses in our systematic review, which shows more problems in these neurodevelopmental domains at long-term follow-up.[6]

Delayed postural control may be a potential early sign for generalized neurodevelopmental disorders later in life.[28] Our results showing a suspect GMF-neurodevelopmental delay at 6 months and a poorer average GMF-neurodevelopmental performance observed at 5 years follow-up, may be a prelude of generalized neurodevelopmental disorders later in time. Milder neurological and neurodevelopmental disorders may manifest later in life.[29,30]

Therefore, both short- (6 months after the infection), middle- (12 months after the infection) and long-term (≥ 24 months after the infection) follow-up will remain important. Early detection of the first signs of neurological sequelae and neurodevelopmental delay helps to refer those with delay for early intervention.

Longitudinal GMF-neurodevelopment

To evaluate the motor neurodevelopment during the first 5 years of life in that time (2008), we had to use different motor neurodevelopment assessment tests. These were the Alberta infant motor scale (AIMS),[23] the Bayley scales of infant and toddler development version-3 (Bayley-3-NL)[31] and the Movement assessment battery for children version-2 (M-ABC-2 NL).[32] This was necessary because at that time no single instrument had been robustly validated to assesses motor performance over such long period of follow-up. Motor neurodevelopment assessment tests are based on different elements, adapted to the age of the child. The AIMS and the Bayley-3-NL have item criteria with a sum score. A higher raw score indicates more mature motor neurodevelopment. The M-ABC-2-NL is based on timed item scores and sum of (in)correct scores. In addition, the motor neurodevelopmental assessment tests had different population reference-norms. The AIMS score is standardized for Canadian children,[23] the Bayley-3-NL[31] and M-ABC-2-NL[32] scores are related to Dutch norms. It is known that Dutch children are slower in GMF-neurodevelopment than Canadian children during the first 15 months of life, but the sequence of achievement of GMF-milestones is the same.[33,34] Dutch children acquire GMF-milestones at an older age. This is resolved by using a *Z*-score of ≤ -1.30 as a cut-off value for suspect GMF-neurodevelopmental delay for the AIMS (in contrast to a *Z*-score of ≤ -1 for the Bayley-3-NL and M-ABC-2-NL).[23,24] Between follow-up moments 6 and 12 months, we saw a positive trend in GMF *Z*-score; on both follow-up times we assessed the motor performance of the children with the AIMS.

Studies based on serial longitudinal assessments of GMF-neurodevelopment in young children with other diagnoses have provided evidence for non-linear motor neurodevelopment. There are intra- and inter-individual variations over time, which resulted in fluctuations in GMF-performance over time.[30,35,36] In our analyses of the serial motor assessments, we used the linear mixed effects models, which incorporate estimates of intra-individual and inter-individual variations over time. It is therefore, a sensitive and valid method to assess motor neurodevelopment longitudinally.[37]

Strengths and limitations

One of the strengths of our study is that only children with RT-qPCR-confirmed PeV in cerebrospinal fluid were included in the PeV-CNS-infection group. The subgroup of children with PeV-infection-elsewhere were those whose paediatrician found no clinical signs of meningitis at presentation or thereafter, and with RT-qPCR positive for PeV in any of the other tested body specimens (this also applies to the children with EV-infection). Neonates do not always show classical clinical signs of CNS-infection. Following procedures from clinical practice, it is usually considered unethical to perform lumbar puncture in children without classical clinical signs of meningitis. Therefore, we cannot completely exclude the possibility that a neonate or young child with PeV-CNS-infection without clinical signs of meningitis at presentation has been misclassified into the PeV-infection-elsewhere group. It is therefore, albeit remotely possible that this may partly explain the small difference in the GMF-neurodevelopmental delay between the group of children with PeV-CNS-infection and with PeV-infection-elsewhere.[38]

Harvala et al. reported that EV-Ribonucleic acid (RNA) is inconsistently detectable in cerebrospinal fluid specimen in children with encephalitis and detectable in cerebrospinal fluid specimen in the majority of children with meningitis.[39] Based on this knowledge, they recommended that positive EV RT-qPCR in both cerebrospinal fluid and another (at least one) body specimen is necessary to confirm the diagnosis meningitis.[39] The children included in our cohort were RT-qPCR tested in cerebrospinal fluid, blood, nasopharyngeal aspirate, urine and faeces.

However, as a result of restriction to children with RT-qPCR-confirmed PeV in cerebrospinal fluid in the case group, the number of our sample of participating children with PeV-CNS-infection is small, which may be seen as a limitation. Still, our recommendation for scientific

research is to choose for a diagnostically homogeneous group. The small power of our studies of children with PeV-CNS-infection is in line with most other follow-up studies in Western countries. To increase the statistical power of follow-up studies, an international consortium could be considered. Currently there is a European Network for Non-Polio Enteroviruses (ENPEN) for a network surveillance and data sharing.[40,41] Unfortunately, data on the short-, middle- and long-term outcomes of PeV-A3-CNS-infection has not been included in the network database.

We recruited the neonates and young children in our study from three general hospitals. This makes extrapolation to tertiary hospitals more difficult. On the other hand, our study sample is probably a better representation of the population of children seen in the majority of general hospitals in most Western countries. That our sample consisted of only Caucasian Dutch children was not by design, however this means that our results cannot be generalized to populations of different races and in other geographic locations.

A strength of our follow-up study is that we assessed the children longitudinally during 5 years of follow-up. This made it possible to take the intra-individual differences of motor neurodevelopment into account. This provides insights for clinicians and neurodevelopmentalists. To our knowledge, none of the cohort studies that prospectively evaluated short-, middle- and long-term motor and cognitive neurodevelopment of children with clinical symptoms of meningitis and positive PeV RT-qPCR in cerebrospinal fluid, have yet performed serial neurodevelopmental assessments.[6,7] A limitation of our serial assessments is that not all the children appeared at each of the follow-up moments. This is a commonly observed problem in lengthy prospective cohort studies. Important to mention is that the missing assessments were not related to the GMF- or FMF-neurodevelopmental outcomes or to the pre-defined risk categories. The reasons for missing follow-up visits were completely at random. The reasons and frequencies did not differ between the case- and control groups. We partially solved this limitation by taking the linear mixed effect models' approach in our analyses which allows incomplete longitudinal data to be considered when estimating model effects. It also has the advantage that the motor assessments do not have to be equally spaced in time.[37]

Clinical implications and further research

GMF-neurodevelopmental delay is assumed to be a good predictor of generalized neurodevelopmental problems in neonates and young children. It is crucial to detect early signs of GMF-neurodevelopmental delay in order to refer children for early intervention. Early intervention will maximize their neurodevelopment. Clinicians and neurodevelopmentalists need to make decisions on frequency and duration of follow-up as well as treatment strategies to mitigate potentially evolving sequelae. With this, we hope our findings will contribute in closing an information gap for clinicians and neurodevelopmentalists.

Despite the clinical relevance of follow-up of neonates and young children with an PeV-CNS-infection, the available knowledge on the sequelae of this virus remains fragmented and incomplete. Many questions are unanswered, such as which neonates and young children are at most risk of neurodevelopment delay. Until now, it has not been scientifically proven that there is an association between the severity at presentation and the overall risk of long-term neurological sequelae or neurodevelopmental delay after PeV-CNS-infection.

To accomplish a more complete follow-up on the outcome of children with PeV-CNS-infection, efforts should be made in different key areas. We have the following recommendations for both research and clinical practice.

Our research recommendations for follow-up studies to determine the causal relationship between PeV-CNS-infection and neurological and neurodevelopmental outcome, are:

- Inclusion of only children with RT-qPCR proven PeV-CNS-infection in cerebrospinal fluid, preferably in combination with a control group.
- Use of clearly defined neurological examination and neurological outcome parameters.
- Use of population norm-referenced neurodevelopment tests with the description of hard outcome measures.
- Serial neurodevelopmental assessment testing, taking into account the intra-individual differences of the neurodevelopment of children over time.
- Sharing and pooling data from different centres to achieve a larger number of children with RT-qPCR proven PeV-CNS-infection in follow-up, for example under the auspices of the European Network for Non-Polio Enteroviruses (ENPEN)[40] or within other international consortia.

Our clinical recommendations for follow-up of neonates and young children PeV-CNS-infection are:

- Both follow-up on short-, middle- and long-term to detect early signs of neurological sequelae and neurodevelopmental delay and to detect generalized neurodevelopmental delay later in life.

Although guidelines for neurodevelopmental follow-up of preterm-born children do exist worldwide, there is presently no protocol for monitoring neurodevelopment in children after PeV-CNS-infection. We believe it is important to develop protocols for monitoring neurodevelopment in neonates and young children after a PeV-CNS-infection. The current thesis provides directions for neurodevelopmental assessment tests, timing and duration of follow-up. These could be implemented in the near future for the follow-up of neonates and young children after an PeV-CNS-infection in daily practice.

Concluding remarks

- Neonates and young children with PeV-CNS-infection show more suspect GMF-neurodevelopmental delay in comparison to the norm-referenced population and to peers with EV-CNS-infection, peers with PeV-infection-elsewhere, EV-infection-elsewhere in the body, and peers in whom no pathogen was detected.
- The GMF-neurodevelopmental outcome reached a clinical threshold in a minority of young children with PeV-CNS-infection.
- In comparison to the population standard norm, the GMF-neurodevelopmental performance of the group of children with PeV-CNS-infection improved between 6 and 24 months but decreased at 5 years.
- The results of our longitudinal follow-up support the concept of serial neurodevelopmental assessments rather than single point assessments.
  Neurodevelopmentalists who practice neurodevelopmental surveillance need to be aware that fluctuations in scoring patterns can be expected in the development of the child.

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8

Summary
Nederlandse samenvatting

Summary

In this thesis, we present various studies that provide insight into the adverse neurologic and neurodevelopmental outcomes of children with parechovirus central nervous system infection (PeV-CNS-infection) in the first 5 years after the infection.

There is a paucity of cohort studies that have prospectively evaluated short- and long-term neurodevelopmental outcome in children with PeV-CNS-infection. We discuss and provide suggestions for follow-up.

PART 1. General introduction

Chapter 1 encloses a general introduction of this thesis, in which the aims and outlines are described. We give an introductory overview on PeV-CNS-infection in neonates and young children.

Introductory overview of PeV-CNS-infection in neonates and young children

Meningitis is an inflammation of the leptomeninges within the subarachnoid space. Encephalitis is an inflammation of the brain parenchyma. If both the leptomeninges and the brain parenchyma are involved, it is called meningoencephalitis. Since these two infections are sometimes difficult to distinguish, we use the term CNS-infection.

Symptoms of meningitis are usually a-specific in neonates and young children. Fever, feeding problems, irritability, respiratory and gastro-intestinal symptoms or a rash are the most frequent presenting symptoms. On physical examination, neck stiffness or a bulging fontanel may be present, but are often absent. Symptoms of encephalitis are more severe, with an altered mental status and focal or diffuse neurological signs, such as photophobia, behavioural changes and focal or generalized convulsions, usually resulting in more severe sequelae than meningitis.

Meningoencephalitis is most commonly caused by bacteria, viruses or parasites. In this thesis, we focus on viral infections. The most common virus is enterovirus (EV) with parechovirus-A (PeV-A) as the second cause.

The clinical presentation of PeV-CNS-infection is similar to that of EV-CNS-infection. It is not possible to differentiate between the two based only on clinical symptoms. The diagnosis of PeV-CNS-infection is based on the combination of medical history and clinical suspicion of CNS-infection, and confirmation by a positive PeV reverse transcriptase quantitative real-time polymerase chain reaction (RT-qPCR).

Accurate incidence and prevalence numbers of PeV-CNS-infection are not well known. The incidence refers to the number of new cases with a specific disease in a specific period. The combination of a scarcity of robust incidence data and the broad spectrum of clinical presentations of neonatal PeV-CNS-infection makes it difficult to determine its real incidence. Most data on PeV-CNS-infection are based on single centre hospital-based studies, which routinely tested for PeV. Prevalence refers to the number of cases who have a specific disease at a specific point in time or during a specific period. PeV-A3 is listed in the distribution of the 15 most reported EV- and PeV-types per year by the United States National Enterovirus Surveillance System, 2014–2016. The prevalence of PeV-A3-CNS-infection is unknown. Since only a couple of years a European Network for Non-Polio Enteroviruses (ENPEN) for surveillance and data sharing has been established.

The PeV belongs to the same family as EV (Picornaviridae). PeV was formerly known as Enterovirus ECHO types E22 and E23. They were reclassified as PeV in the new genus Parechovirus as PeV-A1 and PeV-A2. At present, there are 19 known genotypes of human PeV-A: PeV-A1-19. PeV-B, -C and -D infect only animals.

The more neurotropic strains of PeV-A3 often cause CNS-infection and sepsis like illness in neonates and young children. PeV-A3 strains show a faster replication in neurons and PeV-A3-receptors appear to facilitate entry of the virus through the blood-brain barrier. Once it arrives inside the neuronal cell body, it may result an inhibition of axonal growth and neuronal apoptosis resulting in destruction of the developing brain structures. These effects may result in white and grey matter damage in the brain. The neurologic damage caused by PeV in the CNS may be temporary or permanent. It can subsequently lead to neurological sequelae and neurodevelopmental disorders. These can manifest clinically as neurologic function abnormalities, impairment in visual and auditory functions, gross motor function (GMF) and fine motor function (FMF) neurodevelopmental delay, cognitive impairment, behavioural- and emotional problems and/or speech- and language delay.

With the introduction of RT-qPCR in 2008, the accuracy of diagnosis for PeV improved substantially. It is superior to serology and culture in different body specimens, including cerebrospinal fluid, blood, nasopharyngeal aspirate, urine and faeces. This has made it possible to more accurately distinguish an EV- and PeV-infection and characterize this as a separate clinical entity. Little is known about the long-term neurological and neurodevelopmental outcome of PeV-CNS-infection in neonates and young children.

In this thesis, we present various studies that provide insight into the neurologic and neurodevelopmental outcomes of neonates and young children with a PeV-CNS-infection

in the first 5 years after the infection. We discuss the implications of outcomes and provide suggestions for management and evidence-based follow-up with special emphasis on early detection of GMF-neurodevelopmental delay. Additionally, we critically appraised and analysed existing literature on the neurologic and neurodevelopmental outcomes of neonates and young children with PeV-CNS-infection.

Design of the study on the outcome of neonates and young children with a PeV-CNS-infection

As mentioned earlier, the diagnose of PeV-CNS-infection is based on the combination of medical history and clinical suspicion of CNS-infection, and confirmation by a positive PeV RT-qPCR in the cerebrospinal fluid specimen. All participants were term born children (with a gestational age of more than 37 weeks) and had no co-infections or congenital malformations at the moment of inclusion and during follow-up. In addition, children were included only after prior oral and written informed consent by their parents or legal guardians.

We investigated the association between PeV-CNS-infection and GMF- and FMF-neurodevelopmental outcomes of neonates and young children during a follow-up period of 6 months to 5 years after the infection. We compared the group of children with a PeV-CNS-infection with:

- children with EV-CNS-infection;
- children with PeV-infection-elsewhere in the body (outside the CNS);
- children with EV-infection-elsewhere in the body (outside the CNS);
- a reference group consisting of peers in whom no pathogen was detected (suspected infection);
- population standard norm.

The follow-up moments took place at 6, 12, 24 months and 5 years after the onset of the infection. At each follow-up moment, a pediatrician and pediatric physical therapist screened the children. Prior to the visit parents/legal guardians were instructed not to reveal the study-subgroup to which their child was assigned during the initial study. The independent pediatric physical therapist testing the GMF-neurodevelopment was blinded for the study-subgroups to which the child belonged. Before starting the motor neurodevelopmental assessment each child was scored accordingly as alert, cooperative, actively moving, showing little activity, struggling and/or crying. In case of lack of cooperation, the motor neurodevelopmental assessment was rescheduled on another day. Each child's body mass,

length and head circumference were recorded. Parents/legal guardians were asked to complete a questionnaire on their child's general health, use of medication, presence of recent traumatic head injury and early intervention (particularly pre-speech- and GMF-training).

We assessed the motor neurodevelopment with the *Alberta infant motor scale* (AIMS), the *Bayley scales of infant and toddler development version-3* (Bayley-3-NL and BSID-III) and the *Movement assessment battery for children version-2* (M-ABC-2-NL). We defined an AIMS *Z*-score of ≤ -1.30 (equivalent to the 10th percentile of the AIMS) and a Bayley-3-NL and a M-ABC-2-NL *Z*-score of ≤ -1 as threshold for suspect motor neurodevelopmental delay when we compared the GMF- or FMF-neurodevelopment of participating children to the population standard norm.

PART 2. Literature search Parechovirus CNS-infection and neurologic and neurodevelopmental outcome

In **Chapter 2**, we present a systematic review and meta-analyses of neurodevelopmental outcomes in neonates and young children with PeV-CNS-infection. The primary outcomes are neurologic sequelae, impairment in visual and auditory functions, and GMF-neurodevelopmental delay. The secondary outcomes are signs of late neurodevelopmental delay such as FMF-neurodevelopmental delay, cognitive impairment, behavioural- and emotional problems and speech- and language delay. With this systematic review, we attempted to close the information gap for clinicians and neurodevelopmentalists, on existing literature by conducting a meta-analysis. We hope this will provide a scientific basis to plan frequency and duration of follow-up visits, as well as strategies to mitigate potentially evolving sequelae and neurodevelopmental delay after PeV-CNS-infection.

PART 3. Cross-sectional assessment and assignment to subgroups

Chapter 3 focuses on recruitment, diagnosis, inclusion and exclusion criteria, assignment to subgroups and GMF-neurodevelopmental outcome, 6 months after the infection or suspected infection. We compared the group of children with PeV-CNS-infection with an age of onset ≤ 10 months with peers with a PeV-infection-elsewhere in the body and with peers from the reference group. We tested the GMF-neurodevelopment with the AIMS. The group of children with a PeV-CNS-infection showed a clinically relevant GMF-neurodevelopmental delay compared to the reference group and a suspected GMF-neurodevelopmental delay compared to the population standard norm.

Chapter 4 presents a study in which we compared the GMF- and FMF-neurodevelopmental outcomes in children with an age of onset of 2 days to 12.8 years in subgroups of children with a PeV- and an EV-CNS-infection, PeV- and an EV-infection-elsewhere in the body and a reference group. We tested the GMF- and FMF-neurodevelopment with the BSID-III and the M-ABC-2-NL. We found no statistically significant differences in mean performance GMF- and/or FMF-neurodevelopment and in the number of children with suspected delay in GMF- and/or FMF-neurodevelopment between the PeV- or EV-infected and non-infected children.

PART 4. Longitudinal assessment. The impact of PeV-CNS-infection on gross and fine motor function neurodevelopment

Chapter 5 describes the longitudinal impact of PeV-CNS-infection on GMF-neurodevelopment in neonates and young children. We compared the group of children with PeV-CNS-infection and an age of onset ≤ 24 months with peers from the reference group. The PeV-CNS-infection was serially tested at 6, 12 and 24 months follow-up with the AIMS, the Bayley-3-NL and the M-ABC-2 NL. We performed the analyses longitudinally so that we could take into account the intra- and inter-individual differences of the children. We adjusted for age at onset of the infection, maternal education and time from initial PeV-CNS-infection. We found no statistically significant longitudinal association between PeV-CNS-infection and GMF-neurodevelopment during the first 24 months after the onset of the PeV-CNS-infection. The slope of the GMF-neurodevelopment over 24 months of follow-up of the subgroup of children with a PeV-CNS-infection was slower than that of the reference group. At 6 months follow-up, children with PeV-CNS-infection showed a clinically relevant suspect GMF-neurodevelopmental delay compared to the reference group. This difference was no longer present at 12 and 24 months of follow-up. Adjustment for age at onset weakend the association between PeV-CNS-infection and GMF-neurodevelopment, this was not statistically significant.

Chapter 6 presents the longitudinal impact of PeV-CNS-infection on GMF- and FMF-neurodevelopment in neonates. We compared the group of children with a PeV-CNS-infection with an age of onset ≤ 3 months to peers with an EV-CNS-infection. The GMF-neurodevelopment was serially tested with the AIMS, the Bayley-3-NL and the M-ABC-2-NL at 6, 12 and 24 months and 5 years follow-up. The FMF-neurodevelopment was tested with the Bayley-3-NL and the M-ABC-2-NL at 24 months and 5 years follow-up. We also performed the analyses longitudinally so that we could take into account the

intra- and inter-individual differences of the children. We adjusted for age at onset of the infection, gender, maternal education and time from initial PeV-CNS-infection. We found no statistically significant longitudinal association between the PeV-CNS-infection and GMF-neurodevelopment during the first 5 years after the onset of the PeV-CNS-infection. Our most important finding is that throughout the 5-year follow-up period, children with PeV-CNS-infection with an age of onset ≤ 3 months showed more suspect GMF-neurodevelopmental delay than the population standard norm GMF-score. They also had more suspect GMF-neurodevelopmental delay than peers with an EV-CNS-infection. The average GMF-neurodevelopmental performance of the group of children with PeV-CNS-infection improved between 6 and 24 months. At 5 years of follow-up, the GMF-neurodevelopmental performance decreased.

The FMF-neurodevelopment of the group of children with PeV-CNS-infection was lower at 24 months than that of children with EV-CNS-infection. This normalised with the population standard norm range at 5 year of follow-up.

It is not unthinkable that the poorer average GMF-neurodevelopmental performance at 5 year may be a prelude of generalized neurodevelopmental disorders later in life.

PART 5. General discussion and summary

Chapter 7 contains the general discussion with the conclusions and recommendations of this thesis and **Chapter 8** the English and Dutch summary.

Nederlandse samenvatting

In dit proefschrift beschrijven wij de gevolgen van een parechovirus (PeV) infectie van het centrale zenuwstelsel (CZS) bij pasgeborenen en jonge kinderen op de neurologische ontwikkeling. Dit werd onderzocht gedurende de eerste 5 jaar na het ontstaan van de infectie.

DEEL 1. Algemene inleiding

Hoofdstuk 1 beschrijft een algemene inleiding met de doelstellingen en opzet van de studie naar de uitkomst van pasgeborenen en jonge kinderen met een doorgemaakte PeV-CZS-infectie alsmede een algemeen overzicht van een PeV-CZS-infectie bij pasgeborenen en jonge kinderen.

Algemeen

Meningitis is een ontsteking van de hersenvliezen die de hersenen en het ruggenmerg omhullen in de subarachnoïdale ruimte (leptomeningen). Wanneer daarnaast ook het hersenweefsel betrokken is, spreekt men van meningo-encefalitis. De klinische verschijnselen van meningitis bij pasgeborenen en jonge kinderen zijn meestal aspecifiek. De meest voorkomende verschijnselen zijn koorts, voedingsproblemen, prikkelbaarheid, ademhalings- en gastro-intestinale problemen en/of huiduitslag. Daarnaast kan bij het lichamelijk onderzoek nekstijfheid of een bomberende fontanel worden geobserveerd. De klinische verschijnselen van meningo-encefalitis zijn ernstiger dan van meningitis. Aanvullend op bovenstaande verschijnselen kan er dan ook sprake zijn van een veranderde mentale toestand en/of focale neurologische symptomen, zoals convulsies. Vaak ontbreken deze klinische verschijnselen bij pasgeborenen en jonge kinderen. Omdat meningitis en meningo-encefalitis bij neonaten en jonge kinderen klinisch moeilijk van elkaar te onderscheiden zijn, hebben wij deze samengevoegd en spreken wij in dit proefschrift over een CZS-infectie.

Een CZS-infectie kan veroorzaakt worden door bacteriën, virussen of parasieten. De meest voorkomende virale verwekkers bij pasgeborenen en jonge kinderen zijn enterovirussen (EV) en PeV. De diagnose PeV- en EV-CZS-infectie wordt gesteld op basis van de klinische verschijnselen van een CZS-infectie in combinatie met een positieve PeV- of EV-PCR (polymerase kettingreactie) in de liquor. Sinds 2008 is het mogelijk om het PeV van het EV te onderscheiden door de introductie van een PeV-PCR. Hierdoor is het mogelijk geworden om een PeV-CZS-infectie als een aparte klinische entiteit te onderscheiden en te bestuderen.

Een virale CZS-infectie komt regelmatig voor, maar wordt vaak niet als zodanig in een algemene database geregistreerd door de behandelend arts. Nauwkeurige incidentie- en prevalentiecijfers van PeV-CZS-infecties ontbreken daardoor. Recent is er een Europees netwerk opgericht voor de ontwikkeling van de diagnostiek en het monitoren van gegevens over niet-polio enterovirussen (European Network for Non-Polio Enteroviruses (ENPEN)). Over de neurologische ontwikkelingsgevolgen van een PeV-CZS-infectie bij pasgeborenen en jonge kinderen is nog weinig bekend.

Het PeV behoort tot dezelfde familie als het EV (Picornaviridae). Een (her)classificatie van het menselijke PeV heeft geleid tot het nieuwe genus parechovirus: PeV-A. Momenteel zijn er 19 bekende genotypen: PeV-A1 tot A19. Het PeV-A type 3 (PvA-A3) staat beschreven als een neurotroop virus dat een voorkeur heeft voor het CZS. Als het virus de bloed-hersenbarrière passeert kan de ontstekingsreactie resulteren in schade aan de witte en grijze stof van de hersenen. Hierdoor kan onherstelbare schade aan het hersenweefsel optreden met als gevolg permanente verstoring van de neurologische ontwikkeling. Mogelijke klinische gevolgen hiervan kunnen zijn: tonusregulatieproblemen, visus- en/of gehoorproblemen, achterstand in de grof- en de fijnmotorische ontwikkeling, leerproblemen, gedrags- en emotionele problemen en achterstand in de spraak- en taalontwikkeling.

Opzet van de studie naar de uitkomst van pasgeborenen en jonge kinderen met een doorgemaakte PeV-CZS-infectie

De studie naar de uitkomst van pasgeborenen en jonge kinderen met een doorgemaakte PeV-CZS-infectie beschrijft de werving van kinderen, het stellen van de diagnose, de in- en exclusiecriteria, de toewijzing aan subgroepen, de impact van een PeV-CNS-infectie op de motorische ontwikkeling en de follow-up. Voorwaarden voor de kinderen om aan onze studie te kunnen deelnemen waren de volgende: een blanco medische voorgeschiedenis, een zwangerschapsduur ≥ 37 weken bij geboorte en geen andere bewezen infecties die de oorzaak kunnen zijn van de klinische verschijnselen, zoals neurotrope bacteriële, parasitaire of virale co-infecties. Daarnaast moest vooraf door ouders of verzorgers mondelinge en schriftelijke toestemming zijn gegeven voor het voorgestelde onderzoek en de voorgestelde follow-up. De primaire uitkomstmaat van de huidige follow-up studie was de grofmotorische ontwikkeling, die wordt gezien als een potentieel vroeg-signaal voor een (later zichtbare) algehele ontwikkelingsachterstand (psychomotore retardatie). De groep kinderen met een PeV-CZS-infectie werden in hun motorische ontwikkelingsuitkomst vergeleken met:

- kinderen met een EV-CZS-infectie;
- kinderen met een PeV-infectie elders in het lichaam (buiten het CZS);
- kinderen met een EV-infectie elders in het lichaam (buiten het CZS);
- een referentiegroep bestaande uit leeftijdsgenoten aangemeld met koorts, waarbij met aanvullend laboratoriumonderzoek geen virus of andere ziekteverwekkers zijn aangetoond (vermeende infectie);
- standaardnormwaarden.

De follow-up vond plaats op 4 momenten: 6, 12 en 24 maanden en 5 jaar na het ontstaan van de infectie of de vermeende infectie. Per follow-up moment werd een vragenlijst ingevuld door ouders, vond een medische screening door de kinderarts plaats en werden de motorische ontwikkelingstesten afgenomen door de kinderfysiotherapeut. De kinderfysiotherapeut was geblindeerd voor de diagnose van het kind. De motorische ontwikkeling werd getest met gestandaardiseerde motorische ontwikkelingstesten: de *Alberta infant motor scale* (AIMS), de *Bayley scales of infant and toddler development version-3* (BSID-III en Bayley-3-NL) en de *Movement assessment battery for children version-2* (M-ABC-2-NL). De gebruikte afkapwaarden voor een vermoedelijk vertraagde motorische ontwikkeling tussen de groepen en ten opzichte van de standaardnorm werden respectievelijk als volgt gehanteerd: een verschil tussen de groepen van 1 *Z*-score werd geduid als een klinisch relevant verschil, een *Z*-score ≤ -1,30 op de AIMS en een *Z*-score ≤ -1 op de Bayley-3-NL en de M-ABC-2-NL werden geduid als een vermoedelijke vertraging van de motorische ontwikkeling. Bij gebruik van de BSID-III werden de ruwe scores later omgerekend naar Nederlandse normwaarden.

DEEL 2. Literatuuronderzoek naar neurologische ontwikkelingsgevolgen na een PeV-CZS-infectie

In **Hoofdstuk 2** beschrijven wij een systematische review met meta-analyses. Wij onderzochten de medische literatuur op publicaties over de neurologische ontwikkelingsgevolgen bij pasgeborenen en jonge kinderen met een PeV-CZS-infectie. Deze gevolgen zijn in kaart gebracht door middel van meta-analyses, zowel op korte, middellange als lange termijn. Samenvattend lieten de meta-analyses over de tijd een toename zien van kinderen met neurologische stoornissen (5% in eerste 6 weken en 27% vanaf 12 maanden follow-up). Na 12 maanden bleek op ontwikkelingsgebied dat 9% van de kinderen matige of ernstige problemen hadden in het visuele ontwikkelingsdomein, 19% in het grofmotorische ontwikkelingsdomein, 24% in het cognitieve ontwikkelingsdomein, 23% in de regulatie van gedrag en emoties en 9% in het spraak-taalontwikkelingsdomein. Deze resultaten van de

meta-analyses bevestigen dat op korte termijn neurologische ontwikkelingsgevolgen gedeeltelijk onderkend kunnen worden, maar dat bij jonge kinderen met PeV-CZS-infectie een lange termijn follow-up nodig is om bovengenoemde problematiek nauwkeuriger te kunnen detecteren. Onze aanbeveling aan zorgverleners is om kinderen met een PeV-CZS-infectie langer te volgen, bij voorkeur tot een leeftijd van 5 of 6 jaar.

DEEL 3. Prospectief cross-sectioneel onderzoek

Hoofdstuk 3 focust op de werving van kinderen, het stellen van de diagnose, de in- en exclusiecriteria, de toewijzing aan subgroepen en de grofmotorische ontwikkeling, 6 maanden na de infectie of de vermeende infectie. Wij vergeleken de groep kinderen die ten tijde van het ontstaan van een PeV-CZS-infectie ≤ 10 maanden waren met leeftijdsgenoten met een PeV-infectie elders in het lichaam en met leeftijdsgenoten uit de referentiegroep. De grofmotorische ontwikkeling werd getest met de AIMS. De groep kinderen met een PeV-CZS-infectie liet een klinisch relevante grofmotorische ontwikkelingsvertraging zien ten opzichte van de referentiegroep en een vermoedelijke vertraging ten opzichte van de standaardnormwaarden.

In **Hoofdstuk 4** beschrijven wij de studie waarin de grof- en fijnmotorische ontwikkeling van kinderen die ten tijde van het ontstaan van de PeV- en EV-infectie of vermeende infectie variërend van 2 dagen tot 12,8 jaar op 24 maanden follow-up. Wij onderzochten de groep kinderen met een PeV- en EV-CZS-infectie, de groep kinderen met een PeV- en EV-infectie elders in het lichaam en de referentiegroep. De grof- en fijnmotorische ontwikkeling werd getest met de BSID-III en de M-ABC-2-NL. Wij vonden geen statistisch significante verschillen in gemiddelde prestaties op de grof- en/of fijnmotorische ontwikkeling en geen statistisch significante verschillen in aantal kinderen met een vermoedelijke vertraagde grof- en/of fijnmotorische ontwikkeling tussen de groepen PeV- of EV-geïnfecteerde en niet geïnfecteerde kinderen.

DEEL 4. Prospectief longitudinaal onderzoek

Hoofdstuk 5 beschrijft de longitudinale impact van de PeV-CZS-infectie op de grofmotorische ontwikkeling van pasgeborenen en jonge kinderen. Wij vergeleken de groep kinderen die ten tijde van het ontstaan van een PeV-CZS-infectie ≤ 24 maanden waren met leeftijdsgenoten uit de referentiegroep. De grofmotorische ontwikkeling werd serieel getest op 6, 12 en 24 maanden na het ontstaan van de infectie of de vermeende infectie met de AIMS,

de Bayley-3-NL en de M-ABC-2 NL. De analyse werd longitudinaal uitgevoerd zodat wij rekening konden houden met de intra- en interindividuele verschillen van de kinderen. De statistische correctie was gericht op de leeftijd van ontstaan van de infectie, het opleidingsniveau van de moeder en de tijd verstreken na het ontstaan van de infectie. Met de longitudinale analyse vonden wij geen statistisch significante longitudinale associatie tussen de PeV-CZS-infectie en de grofmotorische ontwikkeling gedurende de eerste 24 maanden na ontstaan van de infectie of de vermeende infectie. De gemiddelde longitudinale grofmotorische ontwikkelingslijn van de groep kinderen met een PeV-CZS-infectie verliep trager dan die van de referentiegroep. Op 6 maanden follow-up was er sprake van een klinisch relevant verschil tussen de groepen, ten nadele van de groep kinderen met een PeV-CZS-infectie. Dit verschil tussen de groepen was niet meer aanwezig op 12 en 24 maanden follow-up. De statistische correctie voor leeftijd van ontstaan van de infectie of vermeende infectie verzwakte de associatie tussen de PeV-CZS-infectie en de grofmotorische ontwikkeling, echter dit was niet statistisch significant.

Hoofdstuk 6 beschrijft de longitudinale impact van de PeV-CZS-infectie op de grof- en fijnmotorische ontwikkeling van pasgeborenen. Wij vergeleken de groep kinderen die ten tijde van het ontstaan van een PeV-CZS-infectie ≤ 3 maanden waren met leeftijdsgenoten met een EV-CZS-infectie. De grofmotorische ontwikkeling werd serieel getest met de AIMS, de Bayley-3-NL en de M-ABC-2 NL op 6, 12 en 24 maanden en 5 jaar na het ontstaan van de infectie en de fijnmotorische ontwikkeling met de Bayley-3-NL en de M-ABC-2 NL op 24 maanden en 5 jaar na het ontstaan van de infectie. De analyse werd longitudinaal uitgevoerd zodat wij rekening konden houden met de intra- en interindividuele verschillen van de kinderen. De statistische correctie was gericht op de leeftijd van ontstaan van de infectie, het opleidingsniveau van de moeder, het geslacht en de tijd verstreken na het ontstaan van de infectie. Met de longitudinale analyse vonden wij geen statistisch significante longitudinale associatie tussen de PeV-CZS-infectie en de grofmotorische ontwikkeling gedurende de eerste 5 jaar na ontstaan van de PeV- of de EV-CZS-infectie. Onze belangrijkste bevinding was dat de groep kinderen met een PeV-CZS-infectie gemiddeld lagere prestaties lieten zien op de grofmotorische ontwikkeling gedurende de gehele follow-up van 5 jaar in vergelijking met de groep leeftijdsgenoten met een EV-CZS-infectie. Ook ten opzichte van de standaardnormwaarden van de grofmotorische ontwikkeling scoorde de groep kinderen met een PeV-CZS-infectie gemiddeld lager dan de groep leeftijdsgenoten met een EV-CZS-infectie. De verschillen waren niet statistisch significant. Tot 24 maanden namen de prestaties op de grofmotorische ontwikkeling toe in zowel de groep kinderen met PeV-CZS-infectie als

de groep kinderen met EV-CZS-infectie. Vervolgens, op 5 jaar follow-up, toonden beide groepen weer een lichte afname in de prestaties op de grofmotorische ontwikkeling. De gemiddelde longitudinale fijnmotorische ontwikkelingslijn van de groep kinderen met een PeV-CZS-infectie verliep trager dan die van de groep kinderen met een EV-CZS-infectie, echter het betrof geen klinisch relevant verschil en het was niet statistisch significant. Op 24 maanden en 5 jaar follow-up vielen de gemiddelde prestaties van beide groepen binnen de normale range van de standaardnormwaarden van de fijnmotorische ontwikkeling. Statistische correcties voor de leeftijd van aanvang van de infectie, het opleidingsniveau van moeder, het geslacht en de tijd sinds het ontstaan van de infectie hadden geen substantiële invloed op het verschil in de grof- en fijnmotorische ontwikkeling tussen de groepen. Omdat de ontwikkeling bij kinderen intra- en interindividuele variaties over tijd kunnen laten zien en daarmee een niet lineair verloop in de motorische ontwikkelingslijnen kunnen vertonen, kan concluderend gesteld worden dat longitudinaal motorisch ontwikkelingsonderzoek een adequate bijdrage kan leveren aan het inzicht van het uiteindelijke functioneren van pasgeborenen en jonge kinderen na het doormaken van een PeV-CZS-infectie.

DEEL 5. Algemene discussie en samenvatting

In **Hoofdstuk 7** volgt de algemene discussie met de conclusie en aanbevelingen van dit proefschrift en in **Hoofdstuk 8** de Engelse en Nederlandse samenvatting.

Addendum

List of abbreviations

AIMS	Alberta infant motor scale
ANOVA	One-way analysis of variance
ASQ-3	Ages and Stages questionnaire version-3
B (beta)	Unstandardized regression coefficient beta
Bayley-3-NL	Bayley scales of infant and toddler development version-3 (NL norms)
BSID-III	Bayley scales of infant and toddler development third version (USA norms)
CI	Confidence interval
CNS	Central nervous system
CP	Cerebral palsy
CSF	Cerebrospinal fluid
cUS	Cranial ultrasound
Df	Degrees of freedom
EEG	Electroencephalography
ENPEN	European Network for Non-Polio Enteroviruses
EV	Enterovirus
EV-71	Enterovirus A type 71
EZ	Elisabeth Hospital
FMF	Fine motor function
GMDS	Griffith mental developmental scale
GMF	Gross motor function
GOS	Glasgow outcome scale
HPeV	Human Parechovirus (abbreviation used prior to 2019)
I^2	Percentage of variability in effect estimates due to heterogeneity
ICU	Intensive care unit (neonatal or paediatric)
IQR	Interquartile range
IVIG	Intravenous immunoglobulin
LOS-f/up	Liverpool outcome score-follow-up
M-ABC-2-NL	Movement assessment battery for children version-2
MRI	Magnetic resonance imaging
NPA	Nasopharyngeal aspirate
NA	Not applicable
NMLE	Package Linear and Nonlinear Mixed-Effects Models R-statistics
No	Number

PeV	Parechovirus (abbreviation used since 2019)
PCR	Polymerase chain reaction
qPCR	Quantitative polymerase chain reaction
RE	Random effects
R-statistics	R Foundation for Statistical computing, Vienna, Austria
RNA	Ribonucleic acid
RT-PCR	Real-time polymerase chain reaction
RT-qPCR	Reverse-transcriptase real time quantitative polymerase chain reaction
SD	Standard deviation of the mean
SPSS	Statistical Package for Social Sciences
ssRNA	Single-stranded Ribonucleinases
TLR	Toll-like receptors
WBC	White blood cell

List of publications

Neurological and neurodevelopmental outcomes after human Parechovirus CNS infection in neonates and young children: a systematic review and meta-analysis

Ted M.T. van Hinsbergh, Roy G. Elbers, J.C.F. Hans Ket, A. Marceline van Furth, Charlie C. Obihara. Lancet Child Adolesc Health. 2020 Aug;4(8):592-605. doi: 10.1016/S2352-4642(20)30181-4. Epub 2020 Jul 22. PMID: 32710840.

Human Parechovirus meningitis and gross-motor neurodevelopment in young children

Ted M.T. van Hinsbergh, Stephanie C.M. de Crom, Robert Lindeboom, A. Marceline van Furth, Charlie C. Obihara. Eur J Pediatr. 2019 Apr;178(4):473-81. doi: 10.1007/s00431-019-03319-6. Epub 2019 Jan 14. PMID: 30637468.

Motor development of children after a human Parechovirus or Enterovirus infection: 24 months follow-up

Stephanie C.M. de Crom, Ted M.T. van Hinsbergh*, Inge A.L.P. Van Beijsterveldt, A. Marceline van Furth, John W.A. Rossen, Charlie C. Obihara. * Joint first authors. Accepted Minerva Pediatrica, April 23 2020.*

Longitudinal association between human Parechovirus central nervous system infection and gross-motor neurodevelopment in young children

Ted M.T. van Hinsbergh, Roy G. Elbers, A. Marceline van Furth, Charlie C. Obihara. Pediatr Infect Dis J. 2019 Feb;38(2):110-4. doi: 10.1097/INF.0000000000002052. PMID: 29601457.

Submitted for publication

Neonatal Parechovirus central nervous system infection: motor neurodevelopmental outcome: a 5-years longitudinal prospective cohort-study

Ted M.T. van Hinsbergh, Roy G. Elbers, Zita Bouman, A. Marceline van Furth, Charlie C. Obihara. Submitted 2021.

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PhD portfolio

PhD candidate	Ted M.T. van Hinsbergh
PhD period	October 2017 – March 2021
PhD supervisors	Prof.dr. A. Marceline Tutu-van Furth
	Dr. Charlie C. Obihara
	Dr. Roy G. Elbers

PhD training	Year	Workload (ECTS)
VUmc Graduate school courses		
Mandatory-Postgraduate Course Advanced Immunology	2019	4.0
Mandatory-Course focused on scientific integrity	2018	2.0
Mandatory-Research ethics for PhD candidates	2019	3.0
GCP: Guidelines and Regulations for medical research	2013/2017	1.0
Scientific Writing in English for Publication in Biomedical Journals	2017	2.0
Course statistics and methodology	2013	3.0
Epidemiologisch onderzoek: opzet en interpretatie (V10)	2013	2.1
Statistical Package for the Social Sciences SPPS I, II and III	2011/2012	2.0
Conference presentations		
Symposium of the Dutch Society of Paediatrics (Nederlandse Vereniging voor Kindergeneeskunde (NVK))	2013	0.71
Symposium of the Dutch Society of Paediatrics (Nederlandse Vereniging voor Kindergeneeskunde (NVK))	2014	0.71
Science day ETZ Scientific poster	2015	0.29
Symposium of the Dutch Society of Paediatrics (Nederlandse Vereniging voor Kindergeneeskunde (NVK))	2017	0.71
Supervising		
Giving education Master Paediatric Physical Therapy	2012	6.00
Other		
International conference on Developmental Coordination Disorder Finland	2019	0.86
International congress Early Intervention	2016	0.57
Minor Neurological Function Kinderneurologisch onderzoek	2017	0.71
Bayley-3 test (measurement used for this study)	2013	0.14
M-ABC-2 test (measurement used for this study)	2010	0.71
Journal club	2017	2.0
Writing van scientific articles	2018/2020	2.0
Personal one-to-one guidance from an experienced researcher PhD conversations 30 hours a year	2017	2.0
Presentation training	2014	0.29

About the author

Ted van Hinsbergh was born long time ago in Helvoirt, the Netherlands. After graduating from the Maurick College Vught in 1977, she obtained her Bachelor's degree in Physiotherapy in 1982. She specialized in Paediatric Physical Therapy in the years thereafter. In 2011 she completed her Master's degree in Specialized Physical Therapy and in 2016 the Master's degree as a clinical epidemiologist. Her Master's theses were on the follow-up of children after a parechovirus and enterovirus central nervous infection. From 2008 until 2016, she conducted the longitudinal neurodevelopmental assessments of the children included in the current thesis, over 5-year. In 2017 she began her PhD degree course. This thesis describes Ted's PhD research on the follow-up of children with parechovirus and enterovirus central nervous infection.

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